**DEPARTMENT OF PLANT PHYSIOLOGY**
**JNKVV -COLLEGE OF AGRICULTURE, JABALPUR AND POWARKHEDA**
**Prepared By:** Dr. A.S. Gontia and Dr. S. K. Pandey

**Course Title: Fundamentals of Crop Physiology**
**Credit: 2 (1+1)**

**B.Sc.(Ag.) Hons. First Year, Second Semester**

**THEORY**

| SNo. | Part to be covered | No. of lectures |
|---|---|---|
| 1. | Introduction to crop physiology and its importance in agriculture. | 01 |
| 2. | Plant Cell: Cell structure and physiological functions of cell wall, cell inclusions. | 01 |
| 3. | Cell organelles and their physiological functions. | 01 |
| 4. | Diffusion of water: Diffusion, osmosis and imbibition, plasmolysis measurements of water status in plants, water potential and its components. | 01 |
| 5. | Absorption of water: Water absorbing system of plant, Kinds of soil water in relation to water absorption. | 01 |
| 6. | Mechanism of water uptake and transport by apoplastic and symplastic methods, factors affecting water absorption. | 01 |
| 7. | Transpiration Mechanism of transpiration, driving force, soil-plant-atmosphere continuum, advantages of transpiration, factors affecting transpiration, anti-transpirants. | 01 |
| 8. | Stomatal physiology: Structure of stomata and mechanism of opening and closing, classification of stomata, theories of mechanism of opening and closing of stomata, bleeding, guttation. | 01 |
| 9. | Mineral nutrition of plants: Criteria of essentiality of elements, essential elements, Physiological role of elements in plants in plants. | 01 |

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| 10. | Method of detection of elements, deficiency symptoms of elements in plants,hydroponics, aeroponincs, nutrient solutions, foliar spray and basal application of nutrients. | 01 |
| 11. | Outer space and apparent free space, theories of active and passive absorption viz; Donnan's equilibrium, contact exchange, carrier concept, ion-exchange or cytochrome pump theory. | 01 |
| 12. | Membrane transporters, aquaporins, mechanism of ion or nutrient uptake and transport in plants, factors affecting nutrient uptake. | 01 |
| 13. | Mechanism of photosynthesis: light reaction, photolysis of water, quantum requirements and pigment systems, photophosphorylation (cycle and non cylclic). | 01 |
| 14. | Calvin cycle, Hatch and Slack pathway. | 01 |
| | **UPTO MID-TERM EXAM** | |
| 15. | CAM pathway,Bacterial photosynthesis, photorespiration, factors affecting rate of photosynthesis. | 01 |
| 16. | Respiration: Glycolysis, Krab's cycle. | 01 |
| 17. | Electron transport system,<br>Pentose phosphate pathway, measurement of respiration, RQ, factors affecting respiration. | 01 |
| 18. | Fat metabolism:-Synthesis of fatty acids. | 01 |
| 19. | Break down of fatty acids. | 01 |
| 20. | Plant growth regulators: Physiological roles and agricultural uses of hormones. | 01 |
| 21. | Auxins, gibberellins, cytokinins. Ethylene, abscisic acid, growth retardants, brassinosteroids, jasmonic acid | 01 |
| 22. | Physiology of crops: Physiological aspects of growth and development of wheat. | 01 |

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| 23. | Physiological aspects of growth and development of rice. | 01 |
| 24. | Physiological aspects of growth and development of maize. | 01 |
| 25. | Physiological aspects of growth and development of soybean. | 01 |
| 26. | Growth analysis: Definitions, phases of growth, factors affecting growth, determinate and indeterminate growth. | 01 |
| 27. | Measurement of growth, growth analytical parameters with formulae. | 01 |
| 28. | Application of growth analysis, role of physiological growth parameters in crop productivity. | 01 |

**Books to be referred**


1. Plant physiology — R.M. Devlin & F.S. Witham (1986)
2. A Text Book of Plant Physiology — P.L. Kochhar & H.N. Krishnamoorthy
3. A Text Book of Plant Physiology — A.S. Gontia
4. Text Book of Plant Physiology — C.P. Malik & A.K. Shrivastava
5. Introductory Plant Physiology — G.Ray Noggle and George. T. Fritz (1994)
6. Text Book of Plant Physiology — S.K. Verma,
7. Crop Physiology — U.S. Gupta
8. Plant Physiology — R.M. Devlin &F. H. Witham
9. Plant Physiology — Frank B. Salisbury and Cleon W. Ross (1995)
10. Plant Physiology — Bernard, S. Meyer and Donald B. Anderson
11. Text Book of Plant Physiology — S. Mukherjee and A.K. Ghosh
12. Plant Physiology — I. Ridge
13. Practical Plant Physiology — O.P. Sharama

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| 14. | Plant Physiology | C.P. Malik |
| 15. | Plant Physiology | S.C. Datta |
| 16. | Plant Physiology | H.S. Shrivastava |
| 17. | Plant Physiology | R.G.S. Bid Well, Macmillan 1979 |
| 18. | An Introduction to Crop Physiology | Milthorpe, F.L. and Moorlely, 1980 |
| 19. | Physiology of Crop Plants | Gardner, T.P., R.B. Pearce and R.B. Mitchell |
| 20. | Introduction to physiology of cereal crops | Shivraj, A., Emkay |

**Course Title - Fundamentals of Crop Physiology**
**Credit- 2 (1+1)**

## 1. LECTURE NOTES

### 1. Introduction to crop physiology and its importance in agriculture.

**Plant Physiology:** The study of natural phenomena in living plants and is concerned with plant **processes, functions** and the **responses in relation to environment**.

**Plant physiology** is a subdiscipline of botany concerned with the functioning, or physiology, of plants. Closely related fields include plant morphology (structure of plants),plant ecology (interaction with the environment), phytochemistry (biochemistry of plants), cell biology, genetics, biophysics and molecular biology.

Plant physiology is the study of vital phenomena in plants. It is the science concerned with processes and functions, the responses of plants to changes in the environment and the growth and development that result from the responses.

**Processes:** Processes mean a natural continuing sequence of events. Examples of processes that occur in plants are photosynthesis, respiration, ion absorption, translocation, stomatal opening and closing, assimilation, transpiration, flowering and seed formation**.**

Fundamental processes such as photosynthesis, respiration, plant nutrition, plant hormone functions, tropisms nastic movements, photoperiodism, photomorphogenesis circadianrhythms,environmentalstress physiology,seed germination, dormancy and stomata fun ction and transpiration,

**Functions:** Function refers to the natural activity of a thing, whether cell, tissue, organ, chemical substance or whatever. Functions are each kind of organ, tissue, cell and cellular organelle in plants and also the function of each chemical constituent, whether ion, molecule or macromolecule.

**Responses in relation to environment**

Although processes and functions are dependent on and modified by such external factors as light and temperature. How processes and functions respond to changes in the environment. Essentially the overall goal of plant physiology is to evolve a detailed and comprehensive knowledge of all the natural phenomena that occur in living plants and thus to understand the nature of plant growth, development and movement.

A **crop** is a plant or animal product that can be grown and harvested extensively for profit or subsistence. Crop may refer either to the harvested parts or to the harvest in a more refined state. Most crops are cultivated in agriculture or aquaculture. A crop may include macroscopic fungus (e.g. mushrooms), or alga (algaculture).

Most crops are harvested as food for humans or fodder for livestock. Some crops are gathered from the wild (including intensive gathering, e.g. ginseng).

Important non-food crops include horticulture, floriculture and industrial crops. Horticulture crops include plants used for other crops (e.g. fruit trees). Floriculture crops include bedding plants, houseplants, flowering garden and pot plants, cut cultivated greens, and cut flowers. Industrial crops are produced for clothing (fiber crops), biofuel (energy crops, algae fuel), or medicine (medicinal plants).

**Crop physiology** is **important in agriculture** as well as horticultural **crops** because: It studies the entire **plant** and **its** communities. They deal with a **plant** in terms of knowledge from the different field such as soil science, **plant physiology**, botany etc. It aims to "increase the yield" of the **plant** economically.

Crop physiology is concerned with the processes and functions of the crops at cellular, sub-cellular and whole plant levels in response to environmental variables and growth**.**

Crop physiology is the study of the plant processes responsible for the growth, development, and production of economic yield by crop plants. Crop physiologists focus on whole plants and plant communities - not individual plant parts, organs, or cells because most of the processes that control yield operate at the whole plant - plant community level. Consequently most crop physiology research is conducted in growth chambers, greenhouses, or in the field.

Crop physiologists investigate processes responsible for the primary productivity of crop communities (e.g., photosynthesis, respiration, light interception, nutrient utilization), how the products of these processes are converted to economic yield (e.g., sink size, seed growth, partitioning, senescence), and developmental processes that define the length of critical growth stages by controlling flowering and maturation. Crop physiology is an integrative science, bringing information from a variety of disciplines (soil science, ecology, plant physiology, botany, statistics, micro meteorology, modeling) to bear on problems of yield improvement and crop management.

In short, physiology is the study of functional aspects of crop plants.

**The role of crop physiology in different aspects of agriculture is discussed here.**

1. Physiological processes by which crops capture resources (light, $CO_2$, water, nutrients) and how crops use these resources to produce profitable products (crop yield & quality).
2. Environmental factors (climate, weather and soil) and crop management factors (genotype, sow date, seed rate, nutrition, agro-chemicals etc.) affect the physiological processes.

3. Crop management affects the environment (e.g. risk of nitrate leaching, Greenhouse Gas [GHG] emissions).

**Early history**

**Sir Francis Bacon** published one of the first plant physiology experiments in 1627 in the book, *Sylva Sylvarum.* Bacon grew several terrestrial plants, including a rose, in water and concluded that soil was only needed to keep the plant upright.

**Jan Baptist van Helmont** published what is considered the first quantitative experiment in plant physiology in 1648. He grew a willow tree for five years in a pot containing 200 pounds of oven-dry soil. The soil lost just two ounces of dry weight and van Helmont concluded that plants get all their weight from water, not soil.

In 1699, **John Woodward** published experiments on growth of **spearmint** in different sources of water. He found that plants grew much better in water with soil added than in distilled water.

**Stephen Hales** is considered the **Father of Plant Physiology** for the many experiments in the 1727 book,*Vegetable Staticks*; though **Julius von Sachs** unified the pieces of plant physiology and put them together as a discipline. His *Lehrbuch der Botanik* was the plant physiology bible of its time.

Researchers discovered in the 1800s that plants absorb essential mineral nutrients as inorganic ions in water. In natural conditions, soil acts as a mineral nutrient reservoir but the soil itself is not essential to plant growth. When the mineral nutrients in the soil are dissolved in water, plant roots absorb nutrients readily, soil is no longer required for the plant to thrive. This observation is the basis for **hydroponics,** the growing of plants in a water solution rather than soil, which has become a standard technique in biological research, teaching lab exercises, crop production and as a hobby.

**Jagadish Chandra Bose**, made pioneering contributions in many fields, like maths, physics, biology, botany, archaeology etc. He invented crescograph for measuring **plant** growth. He is generally regarded as **Father of Indian Plant Physiology**.

**Importance in agriculture:**

**Photosynthesis**:

Through photosynthesis green plants are able to manufacture their food themselves. Certain plants like maize, sugarcane and sorghum possess C4 pathway which have higher adaptability to drought, high temperature and high light intensity. Such plants also lacking photorespiration. Therefore, there is a great need to reduce photorespiration through breeding program. The yields of many crops are poor due to poor translocation of photosynthates to the sink. Therefore, there is an urgent need to identify the varieties with better translocation of photosynthates towards reproductive parts.

**Mineral nutrition:**

There is an urgent need to identify the fertilizer requirements, mineral application and proper stage of application. Application of excess nitrogenous fertilizers in cotton resulted in decreased yield. In pulses application of nitrogenous fertilizers at an early stages inhibits the development of nodules leading to poor yield. In later stages, at flowering nodule activity decreases, this is the time for proper application of nitrogen. Through hydroponics plants can be grown without soil. Through foliar application many elements which gets precipitated are made available. There is an urgent need to identify the nodule activity, survival period etc. The efforts are being made to transfer nitrogen fixing genes (nif genes) in cereals also so that cereals could also be able to fix atmospheric nitrogen. It has been identified through physiological research that during pod development the nodule activity is very slow. Therefore, application of nitrogenous fertilizers during this time is a must.

**Stress Physiology**:

Plants absorb large quantities of water and 98% is lost through transpiration. Therefore, there is need to check the excessive loss by using antitranspirants. Efforts are being made to identify the drought resistant varieties. Critical stages have to be identified where maximum loss may occur due to water stress. Plant responses to environmental extremes such as excess or deficiency of water , mineral salts , high and low temperature and atmospheric pollutants have been worked out.

**Improvement of plant type:**

Indeterminate cotton varieties are not suitable for mechanical harvesting. Therefore, by using growth retardants such as CCC such varieties can be made compact which would facilitate easy harvesting. Lodging in tall varieties of wheat can also be checked by spraying growth retardants. Many tall trees can be grown in pots. In grapes ring of bark (phloem) above soil level is removed which cuts down the translocation of photosynthates to the underground parts. Consequently, the fruits become bigger in size.

**Plant growth substances**:

Use of growth regulators change the plant architecture, make the plants to bloom, hasten the rooting of stem cuttings and ripening of fruits, extend the shelf life of cut flowers, vegetables and fruits.

**Rooting of cuttings**:

Many fruits and timber yielding plants are slow growing when propagated through seeds. Such plants do not resemble the parent trees in vigour and quality of fruits. This problem can be overcome by dipping the stem cuttings in 100 to 500 ppm of synthetic auxins like NAA and IBA. Seridax is a commercially available auxin mixture which is used extensively.

**Breaking of dormancy**:

Seeds of some species of grapes, apple and peach remain dormant and do not germinate till they pass through winter. Soaking of seeds in GA breaks the dormancy of such seeds and such seeds can germinate soon after harvesting.

**Inhibition of sprouting**:

Potato tubers and onion bulbs sprout when stored which leads to loss of weight and deterioration of quality. This can be prevented by treating the tubers with methyl ester of NAA (MENA) which is an auxin and onion bulbs with melic hydrazide (MH).

**Controlling the size of the plant:**

In sugarcane GA is used to increase the length of internode and sugar content. Many ornamentals are grown by using growth retardants like Cycocel, B-Nine and Phosphon D in pots. In tobacco flowering leads to emergence of suckers (short lateral branches) due to this quality of tobacco leaf is deteriorated. This problem can be overcome by using MH.

**Promotion of flowering**

Application of NAA causes uniform flowering in pineapple leading to development of uniform sized fruits. Recently ethephon (ethrel) is used for this purpose in pineapple orchards. This is also used to increase the number of female flowers and consequently yield. Normally in cucurbits the flowers are unisexual and yield is limited by number of female flowers produced by the plant.

**Control of abscission:**

In apple, mango and others fruit falls before maturity causing reduction in yield. This is prevented by spraying NAA and 2, 4–D which are used to prevent pre harvest fall in citrus fruits.

**Fruit development and ripening**:

Normally ovary after pollination form fruits and ovules seed. Sometimes due to non formation of pollen grains fertilization is failed and ovary withers and falls down. In tomato this can be checked by using Para chloro phenoxy acetic acid (PCPA) .This also helps in fruit setting without fertilization. This is called parthenocarpic fruit development. Artificial ripening in mango, banana and oranges can be induced by spraying the fruits with calcium carbide which releases acetylene and ethephon which releases the ethylene.

**Weed control:**

Synthetic auxin like 2, 4-D (2,4 Dichloro phenoxy acetic acid) when sprayed kills dicot weeds like chenopodium. They act on living cells of vascular ray cells, increase the respiratory rate leading to death of cells.

Following classes of herbicides :

1. Phenoxy compounds Ex. 2,4-D, 2,4-5 T.
2. Triazines Ex. Simazine and Atrazine.
3. Substituted ureas Ex. Monuron and Diuron.
4. Carbonates Ex. Barban.

**Tissue culture:**

Through tissue culture technique plants can be developed from tissues like parenchyma, phloem and pollen in synthetic media. This technique is employed to raise the haploid plants through pollen culture.

**Other than this importance in crop physiology:**

1. Seed physiology
2. Optimum seedling growth and Plant population
3. Growth measurement of the crops
4. Harvest Index
5. Transpiration efficiency
6. Water use efficiency (WUE)
7. Nitrogen use efficiency(NUE)
8. Post harvest physiology.

## 2. LECTURE NOTES

### 2. Plant Cell: Cell Structure and Physiological Functions of Cell Wall, Cell Inclusions. Cell organelles and their physiological functions.

**Plant Cell :**

The term cell is derived from the Latin cella, meaning storeroom or chamber. It was first used in biology in 1665 by the English botanist Robert Hooke to describe the individual units of the honeycomb-like structure he observed in cork under a compound microscope. The “cells” Hooke observed were actually the empty lumens of dead cells surrounded by cell walls, but the term is an apt one because cells are the basic building blocks that define plant structure.

As Earth’s primary producers, green plants are the ultimate solar collectors. They harvest the energy of sunlight by converting light energy to chemical energy, which they store in bonds formed when they synthesize carbohydrates from carbon dioxide and water.

• Other than certain reproductive cells, plants are nonmotile. As a substitute for motility, they have evolved the ability to grow toward essential resources, such as light, water, and mineral nutrients, throughout their life span.

• Terrestrial plants are structurally reinforced to support their mass as they grow toward sunlight against the pull of gravity.

• Terrestrial plants lose water continuously by evaporation and have evolved mechanisms for avoiding desiccation.

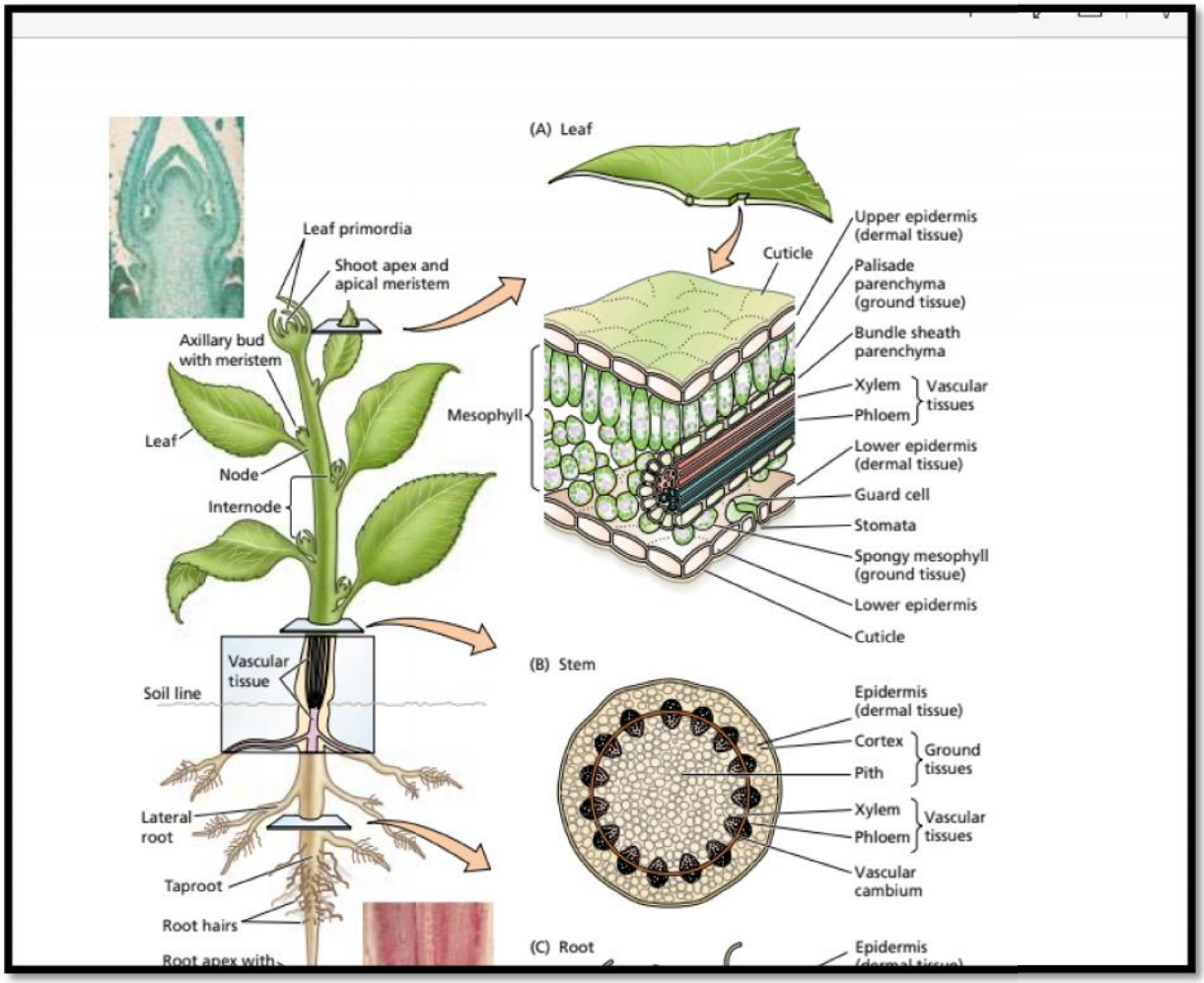
• Terrestrial plants have mechanisms for moving water and minerals from the soil to the sites of photosynthesis and growth, as well as mechanisms for moving the products of photosynthesis to nonphotosynthetic organs and tissues.

**OVERVIEW OF PLANT :**

The vegetative body is composed of three organs: **leaf, stem, and root**. The primary function of a leaf is photosynthesis, that of the stem is support, and that of the root is anchorage and

absorption of water and minerals. Leaves are attached to the stem at nodes, and the region of the stem between two nodes is termed the internode. The stem together with its leaves is commonly referred to as the shoot. There are two categories of seed plants: gymnosperms (from the Greek for “naked seed”) and angiosperms (based on the Greek for “vessel seed,” or seeds contained in a vessel). Gymnosperms are the less advanced type; about 700 species are known. The largest group of gymnosperms is the conifers (“cone-bearers”), which include such commercially important forest trees as pine, fir, spruce, and redwood. Angiosperms, the more advanced type of seed plant, first became abundant during the Cretaceous period, about 100 million years ago. Today, they dominate the landscape, easily outcompeting the gymnosperms. About 250,000 species are known (Fig. 1.2).

**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.



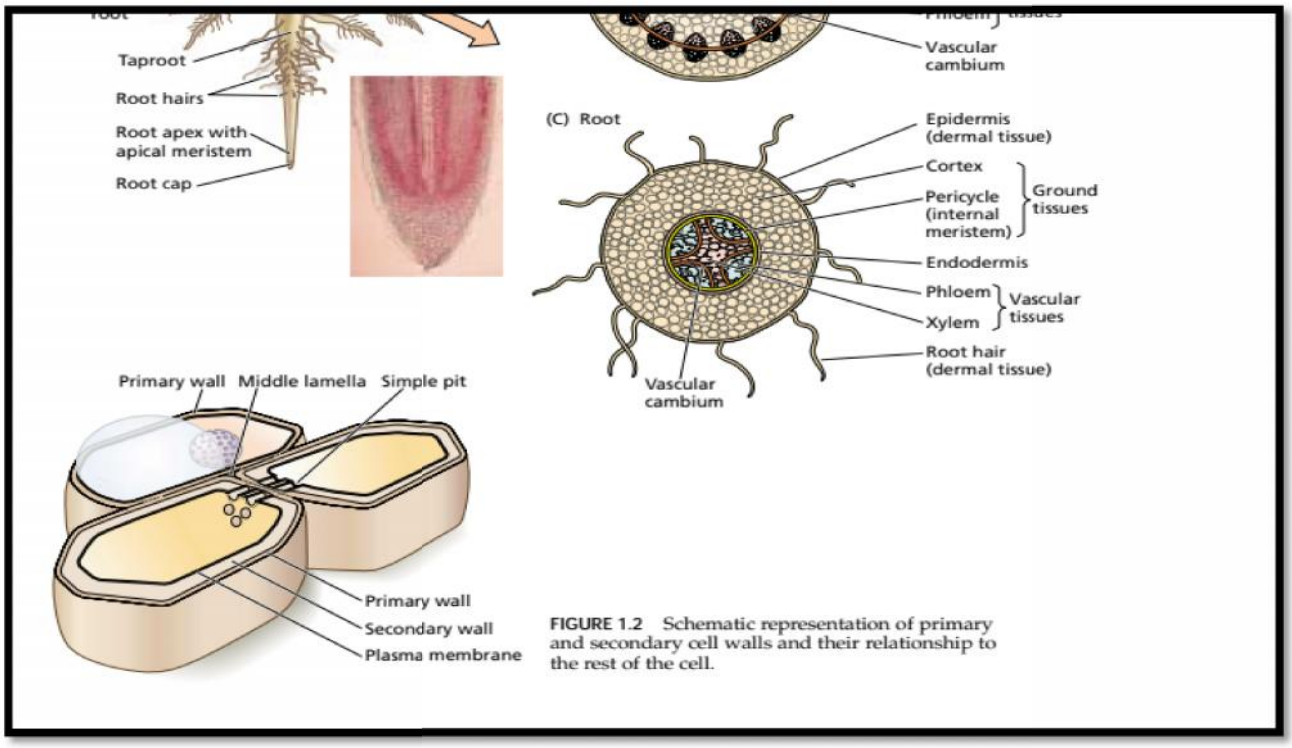



FIGURE 1.2 Schematic representation of primary and secondary cell walls and their relationship to the rest of the cell.

**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

**THE PLANT CELL**

Plants are multicellular organisms composed of millions of cells with specialized functions. At maturity, such specialized cells may differ greatly from one another in their structures. However, all plant cells have the same basic eukaryotic organization: They contain a nucleus, a cytoplasm, and subcellular organelles, and they are enclosed in a membrane that defines their boundaries (Figure 1.4). Certain structures, including the nucleus, can be lost during cell maturation, but all plant cells begin with a similar complement of organelles.

. **Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

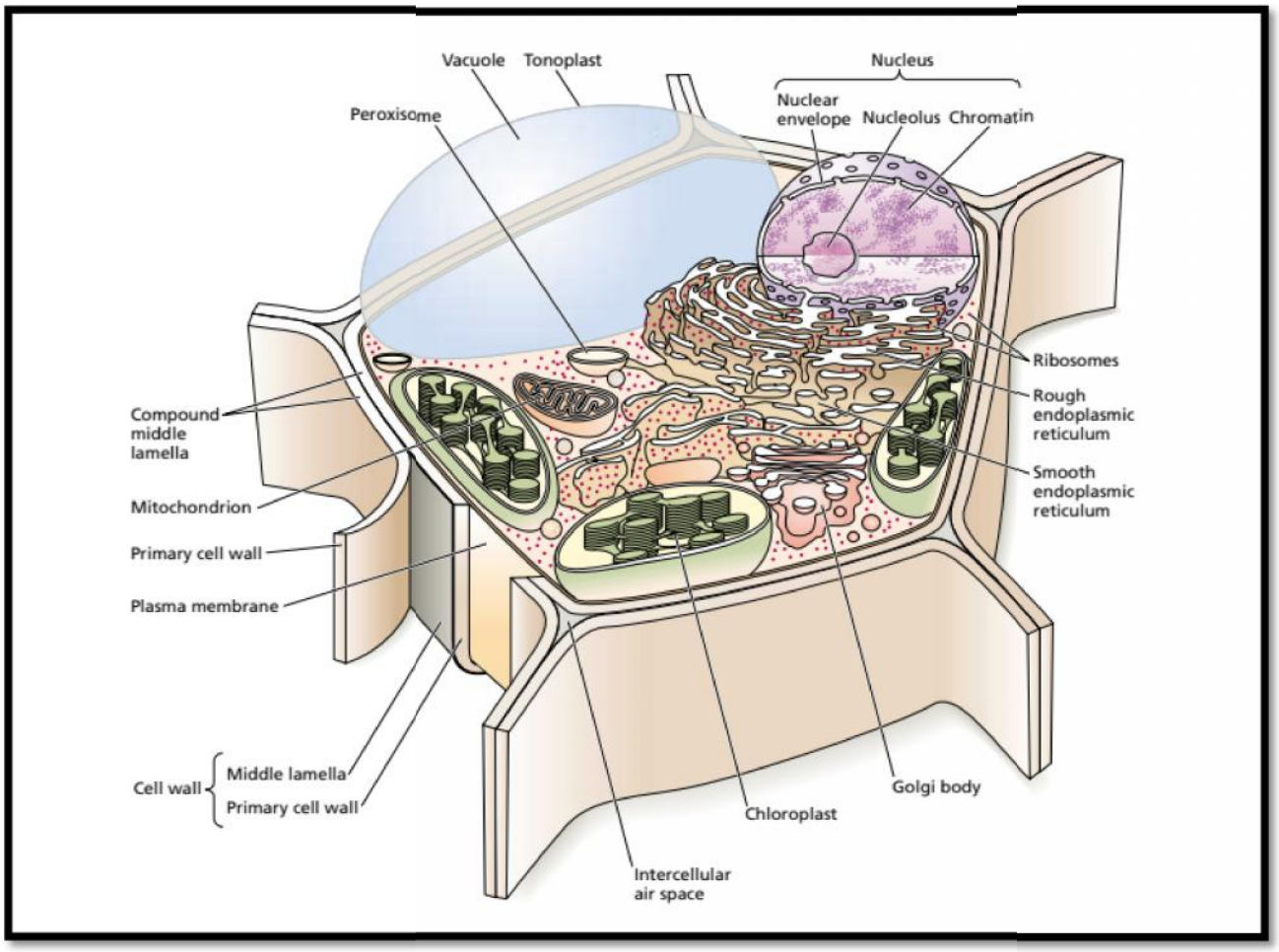



**Fig 1.2** Diagrammatic representation of a plant cell.

**Biological Membranes Are Phospholipid Bilayers That Contain Proteins**

All cells are enclosed in a membrane that serves as their outer boundary, separating the cytoplasm from the external environment. This plasma membrane (also called plasmalemma) allows the cell to take up and retain certain substances while excluding others. Various transport proteins embedded in the plasma membrane are responsible for this selective traffic of solutes across the membrane. The accumulation of ions or molecules in the cytosol through the action of transport proteins consumes metabolic energy. Membranes also delimit the boundaries of the

specialized internal organelles of the cell and regulate the fluxes of ions and metabolites into and out of these compartments. According to the fluid-mosaic model, all biological membranes have the same basic molecular organization. They consist of a double layer (bilayer) of either phospholipids or, in the case of chloroplasts, glycosylglycerides, in which proteins are embedded (Figure 1.5A and B). In most membranes, proteins make up about half of the membrane's mass. However, the composition of the lipid components and the properties of the proteins vary from membrane to membrane, conferring on each membrane its unique functional characteristics.

**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

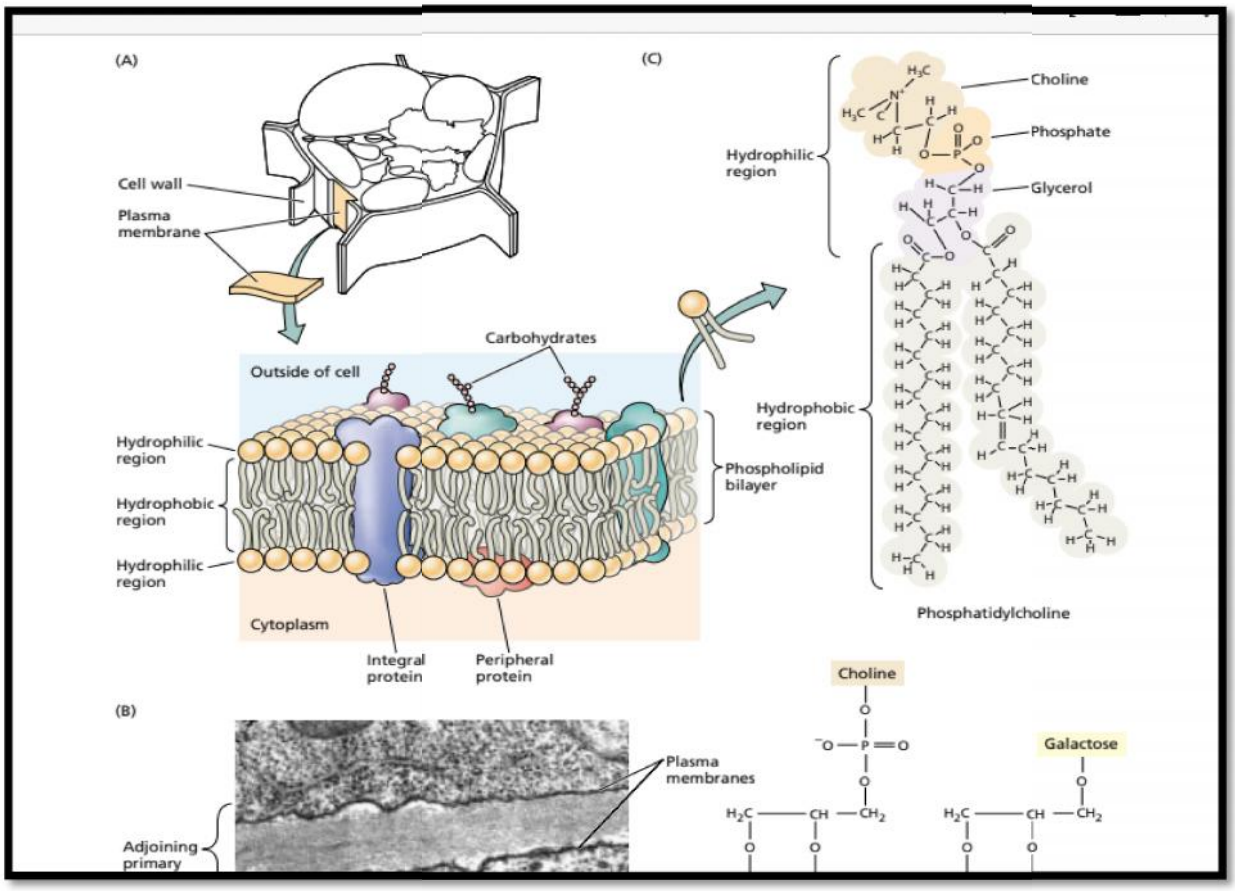



**FIG.1.3** (A) The plasma membrane, endoplasmic reticulum, and other endomembranes of plant cells consist of proteins embedded in a phospholipid bilayer. (B) This transmission electron micrograph shows plasma membranes in cells from the meristematic region of a root tip of cress (Lepidium sativum).

**The Nucleus Contains Most of the Genetic Material of the Cell**

The nucleus (plural nuclei) is the organelle that contains the genetic information primarily responsible for regulating the metabolism, growth, and differentiation of the cell. Collectively, these genes and their intervening sequences are referred to as the nuclear genome. The size of the nuclear genome in plants is highly variable, ranging from about 1.2 × 108 base pairs for the diminutive dicot Arabidopsis thaliana to 1 × 1011 base pairs for the lily Fritillaria assyriaca.

The nucleus is surrounded by a double membrane called the nuclear envelope.The space between the two membranes of the nuclear envelope is called the perinuclear space, and the two membranes of the nuclear envelope join at sites called nuclear pores. The nuclear "pore" is actually an elaborate structure composed of more than a hundred different proteins arranged octagonally to form a nuclear pore complex. There can be very few to many thousands of nuclear pore complexes on an individual nuclear envelope. The central "plug" of the complex acts as an active (ATPdriven) transporter that facilitates the movement of macromolecules and ribosomal subunits both into and out of the nucleus A specific amino acid sequence called the nuclear localization signal is required for a protein to gain entry into the nucleus. The nucleus is the site of storage and replication of the chromosomes, composed of DNA and its associated proteins. Collectively, this DNA–protein complex is known as chromatin. The linear length of all the DNA within any plant genome is usually millions of times greater than the diameter of the nucleus in which it is found. To solve the problem of packaging this chromosomal DNA within the nucleus, segments of the linear double helix of DNA are coiled twice around a solid cylinder of eight histone protein molecules, forming a nucleosome. Nucleosomes are arranged like beads on a string along the length of each chromosome. During mitosis, the chromatin condenses, first by coiling tightly into a 30 nm chromatin fiber, with six nucleosomes per turn, followed by further folding and packing processes that depend on interactions between proteins and nucleic acids. At interphase, two types of chromatin are visible: heterochromatin and euchromatin. About 10% of the DNA consists of heterochromatin, a highly compact and transcriptionally inactive form of chromatin. The rest of the DNA consists of euchromatin, the dispersed, transcriptionally active form. Only about 10% of the euchromatin is transcriptionally active at any given time. The remainder exists in an intermediate state of condensation, between heterochromatin and transcriptionally active euchromatin. Nuclei contain a densely granular region, called the nucleolus (plural nucleoli), that is the site of ribosome synthesis. The nucleolus includes portions of one or more chromosomes where ribosomal RNA (rRNA) genes are clustered to form a structure called the nucleolar organizer. Typical cells have one or more nucleoli per nucleus. Each 80S ribosome is made of a large and a small subunit, and each subunit is a complex aggregate of rRNA and specific proteins. The two subunits exit the nucleus separately, through the nuclear pore, and then unite in the cytoplasm to form a complete ribosome.

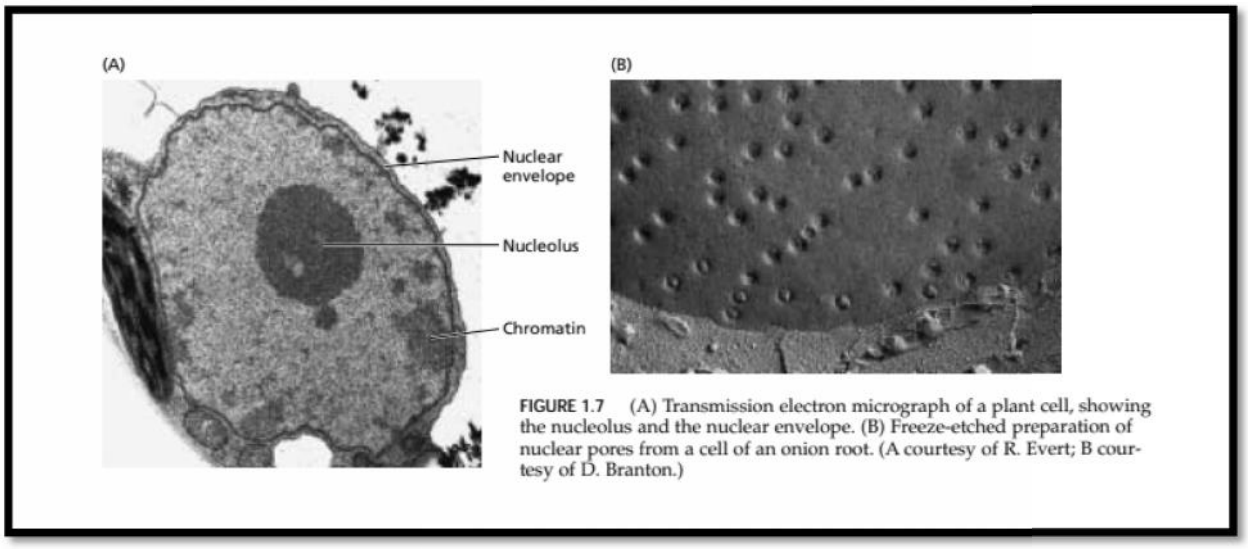
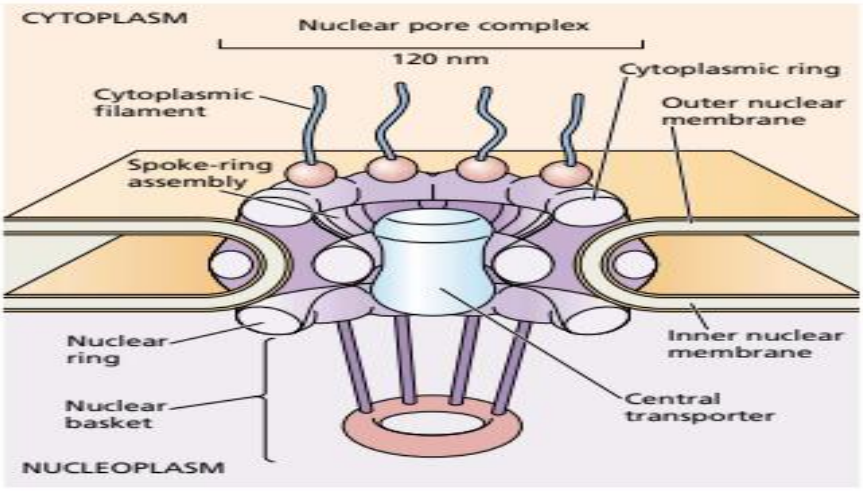



FIGURE 1.7 (A) Transmission electron micrograph of a plant cell, showing the nucleolus and the nuclear envelope. (B) Freeze-etched preparation of nuclear pores from a cell of an onion root. (A courtesy of R. Evert; B courtesy of D. Branton.)

**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.




**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

**FIGURE 1.4** Schematic model of the structure of the nuclear pore complex. Parallel rings composed of eight subunits each are arranged octagonally near the inner and outer membranes of the nuclear envelope. Various proteins form the other structures, such as the nuclear ring, the spokering assembly, the central transporter, the cytoplasmic filaments, and the nuclear basket.

**The Endoplasmic Reticulum Is a Network of Internal Membranes Cells** have an elaborate network of internal membranes called the endoplasmic reticulum (ER). The membranes of the ER are typical lipid bilayers with interspersed integral and peripheral proteins. These membranes form flattened or tubular sacs known as cisternae (singular cisterna). Ultrastructural studies have shown that the ER is continuous with the outer membrane of the nuclear envelope. There are two types of ER—smooth and rough and the two types are interconnected. Rough ER (RER) differs from smooth ER in that it is covered with ribosomes that are actively engaged in protein synthesis; in addition, rough ER tends to be lamellar (a flat sheet composed of two unit membranes), while smooth ER tends to be tubular, although a gradation for each type can be observed in almost any cell. The structural differences between the two forms of ER are accompanied by functional differences. Smooth ER functions as a major site of lipid synthesis and membrane assembly. Rough ER is the site of synthesis of membrane proteins and proteins to be secreted outside the cell or into the vacuoles. Secretion of Proteins from Cells Begins with the Rough ER Proteins destined for secretion cross the RER membrane and enter the lumen of the ER.

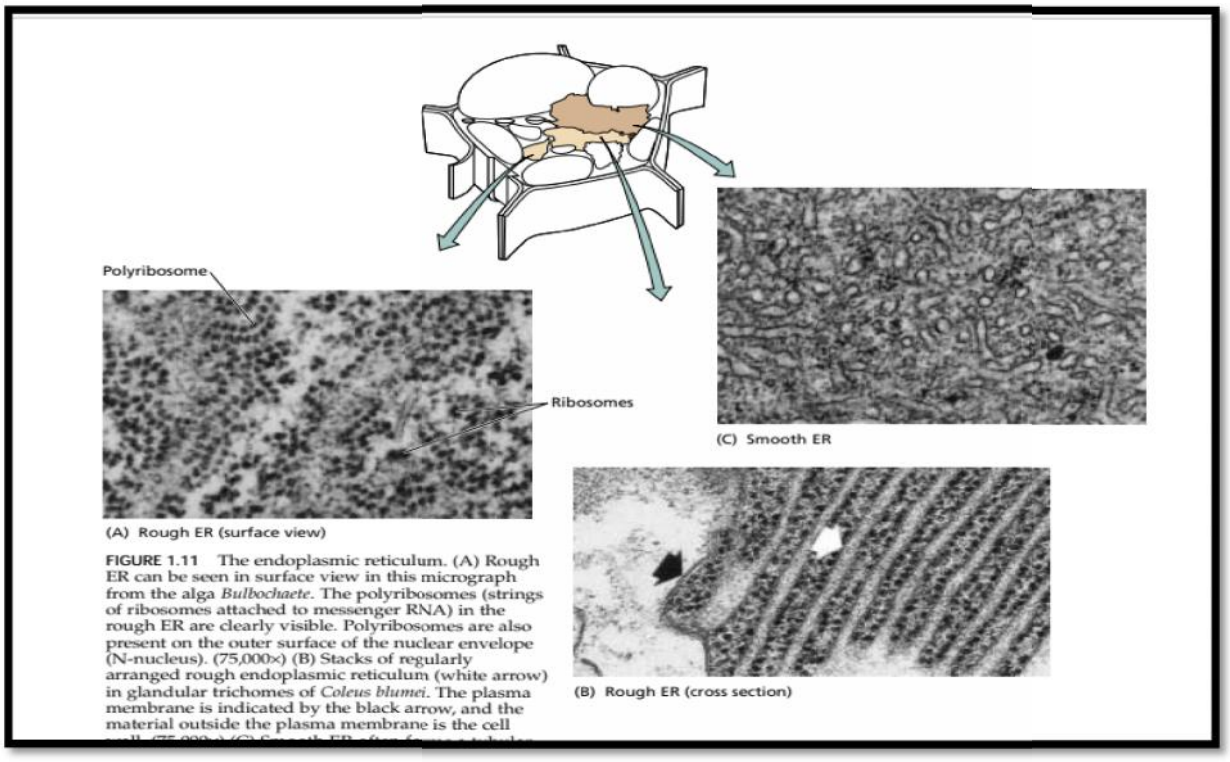
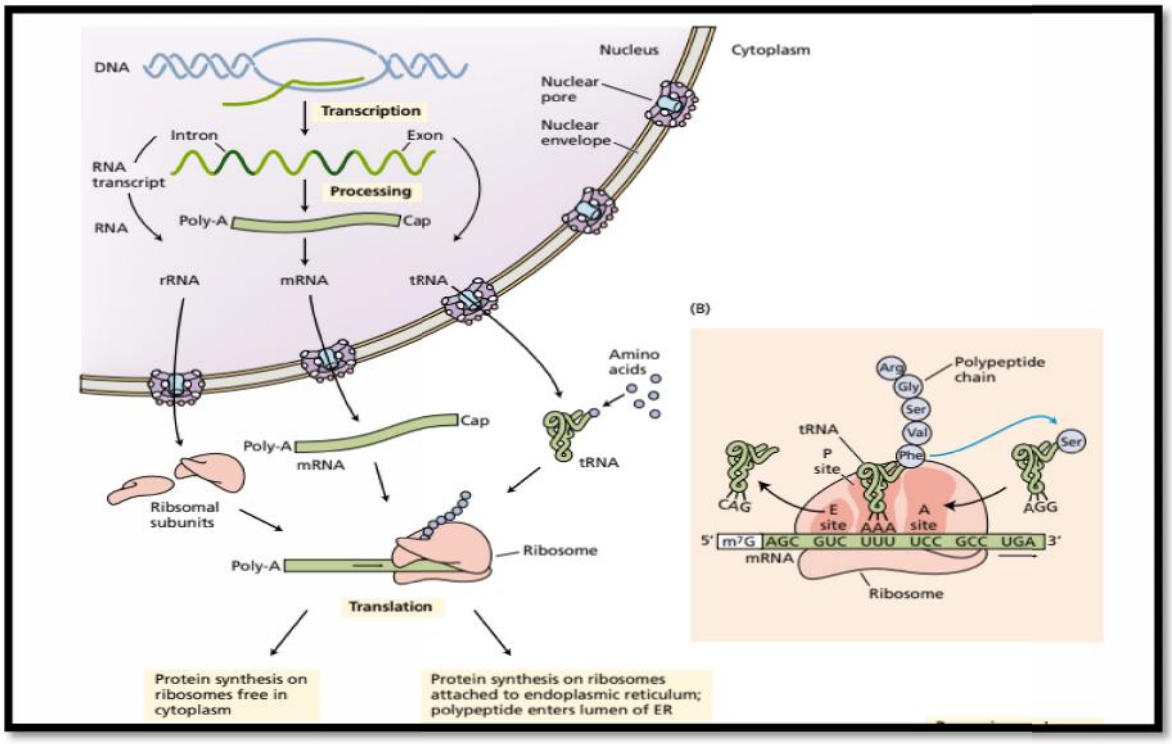
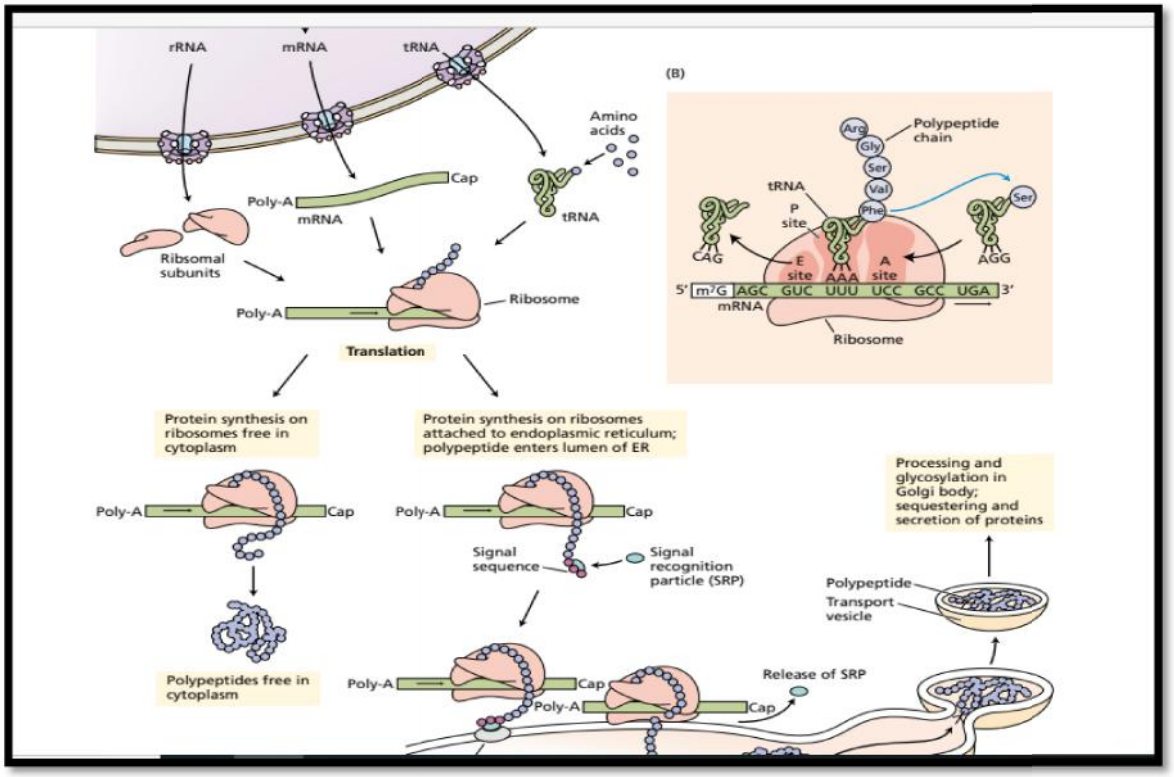



FIGURE 1.11 The endoplasmic reticulum. (A) Rough ER can be seen in surface view in this micrograph from the alga *Bulbochaete*. The polyribosomes (strings of ribosomes attached to messenger RNA) in the rough ER are clearly visible. Polyribosomes are also present on the outer surface of the nuclear envelope (N-nucleus). (75,000×) (B) Stacks of regularly arranged rough endoplasmic reticulum (white arrow) in glandular trichomes of *Coleus blumei*. The plasma membrane is indicated by the black arrow, and the material outside the plasma membrane is the cell

**Secretion of Proteins from Cells Begins with the Rough ER :** Proteins destined for secretion cross the RER membrane and enter the lumen of the ER. This is the first step in the sequence that is complementary to a specific gene. The RNA transcript is processed to become messenger

RNA (mRNA), which moves from the nucleus to the cytoplasm. The mRNA in the cytoplasm attaches first to the small ribosomal subunit and then to the large subunit to initiate translation.



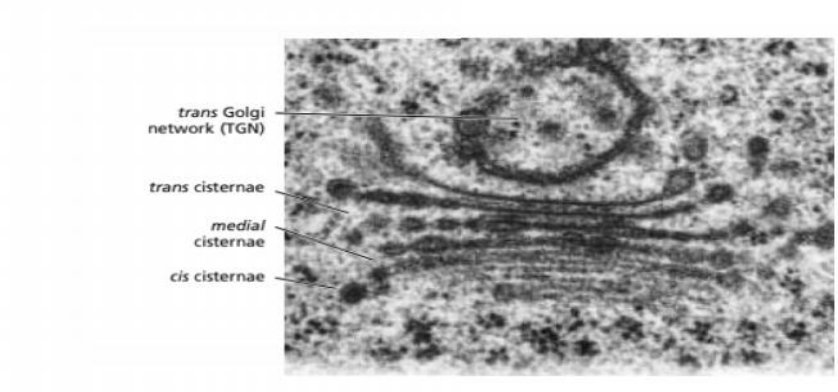



**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

**Proteins and Polysaccharides for Secretion Are Processed in the Golgi Apparatus**

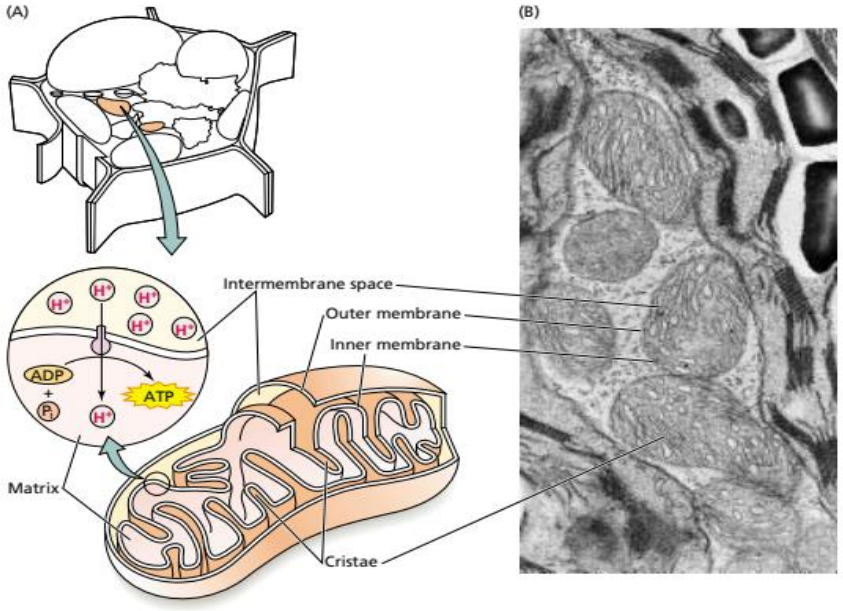
The Golgi apparatus (also called Golgi complex) of plant cells is a dynamic structure consisting of one or more stacks of three to ten flattened membrane sacs, or cisternae, and an irregular network of tubules and vesicles called the trans Golgi network (TGN) (see Figure 1.12). Each individual stack is called a Golgi body or dictyosome. The Golgi body has distinct functional regions: The cisternae closest to the plasma membrane are called the trans face, and the cisternae closest to the center of the cell are called the cis face. The medial cisternae are between the trans and cis cisternae. The trans Golgi network is located on the trans face. The entire structure is stabilized by the presence of intercisternal elements, protein crosslinks that hold the cisternae together. Whereas in animal cells Golgi bodies tend to be clustered in one part of the cell and are interconnected via tubules, plant cells contain up to several hundred apparently separate Golgi bodies dispersed throughout the cytoplasm (Driouich et al. 1994). The Golgi apparatus plays a key role in the synthesis and secretion of complex polysaccharides (polymers composed of different types of sugars) and in the assembly of the oligosaccharide side chains of glycoproteins (Driouich et al. 1994). As noted already, the polypeptide chains of future glycoproteins are first synthesized on the rough ER, then transferred across the ER membrane, and glycosylated on the —NH2 groups of asparagine residues. Further modifications of, and additions to, the oligosaccharide side chains are carried out in the Golgi. Glycoproteins destined

for secretion reach the Golgi via vesicles that bud off from the RER. The exact pathway of glycoproteins through the plant Golgi apparatus is not yet known.




**The Central Vacuole Contains Water and Solutes** Mature living plant cells contain large, water-filled central vacuoles that can occupy 80 to 90% of the total volume of the cell (see Figure 1.4). Each vacuole is surrounded by a vacuolar membrane, or tonoplast. Many cells also have cytoplasmic strands that run through the vacuole, but each transvacuolar strand is surrounded by the tonoplast.

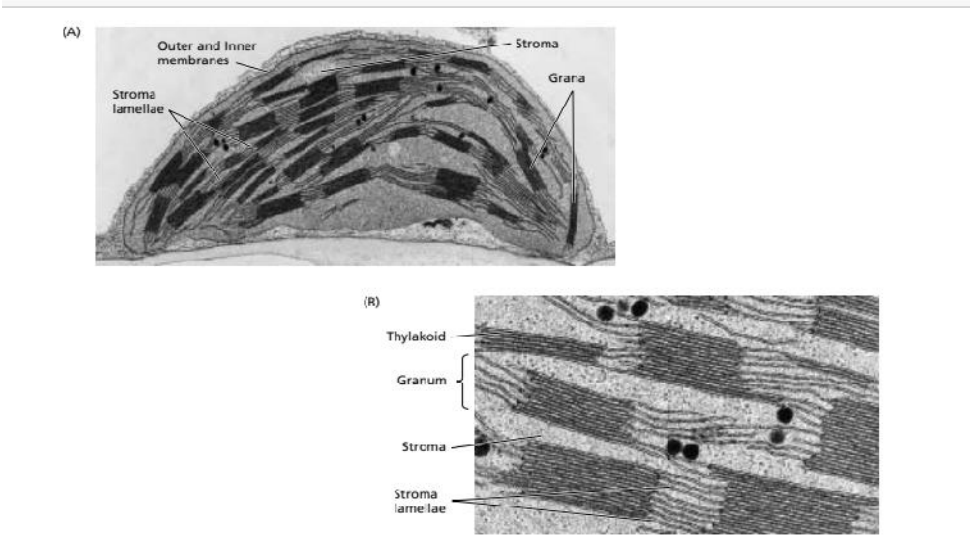
**Mitochondria and Chloroplasts Are Sites of Energy Conversion :** A typical plant cell has two types of energy-producing organelles: mitochondria and chloroplasts. Both types are separated from the cytosol by a double membrane (an outer and an inner membrane). Mitochondria (singular mitochondrion) are the cellular sites of respiration, a process in which the energy released from sugar metabolism is used for the synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and inorganic phosphate (Pi ). Mitochondria can vary in shape from spherical to tubular, but they all have a smooth outer membrane and a highly convoluted inner membrane. The infoldings of the inner membrane are called cristae (singular crista). The compartment enclosed by the inner membrane, the mitochondrial matrix, contains the enzymes of the pathway of intermediary metabolism called the Krebs cycle. In contrast to the mitochondrial outer membrane and all other membranes in the cell, the inner membrane of a mitochondrion is almost 70% protein and contains some phospholipids that are unique to the organelle (e.g., cardiolipin). The proteins in and on the inner membrane have special enzymatic and transport capacities. The inner membrane is highly impermeable to the passage of H+; that is, it serves as a barrier to the movement of protons. This important feature allows the formation of electrochemical gradients. Dissipation of such gradients by the controlled movement of H+ ions through the transmembrane enzyme ATP synthase is coupled to the phosphorylation of ADP to produce ATP. ATP can then be released to other cellular sites where energy is needed to drive specific reactions.

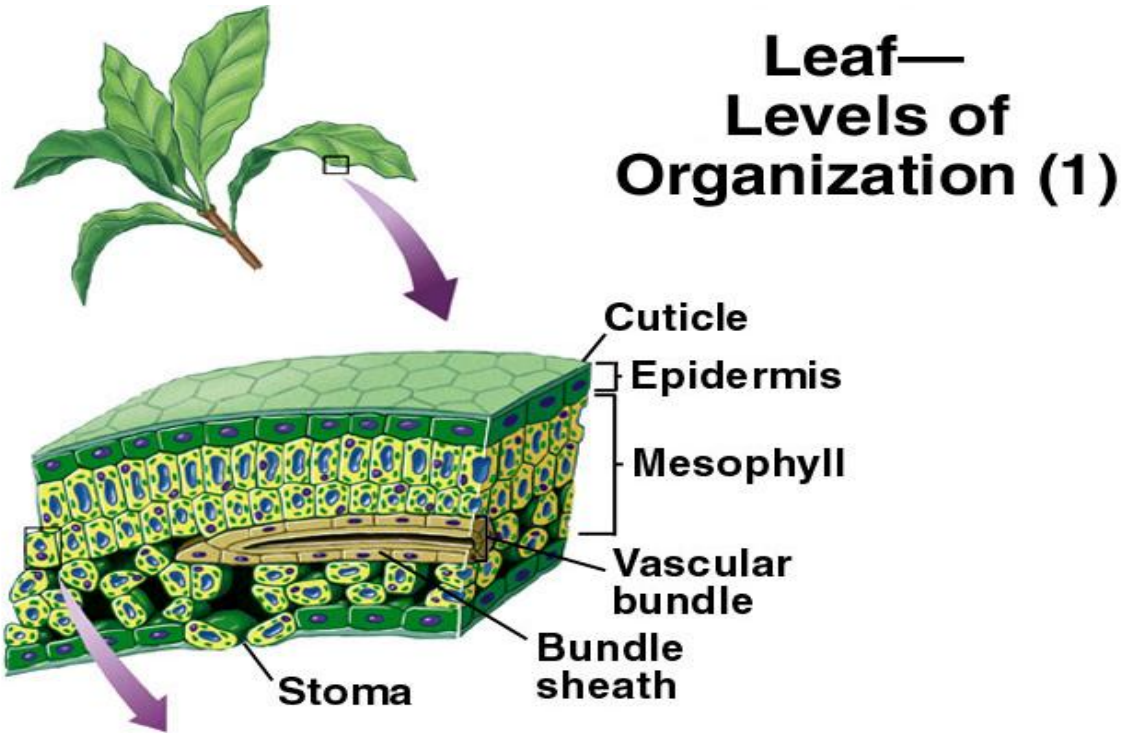



**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

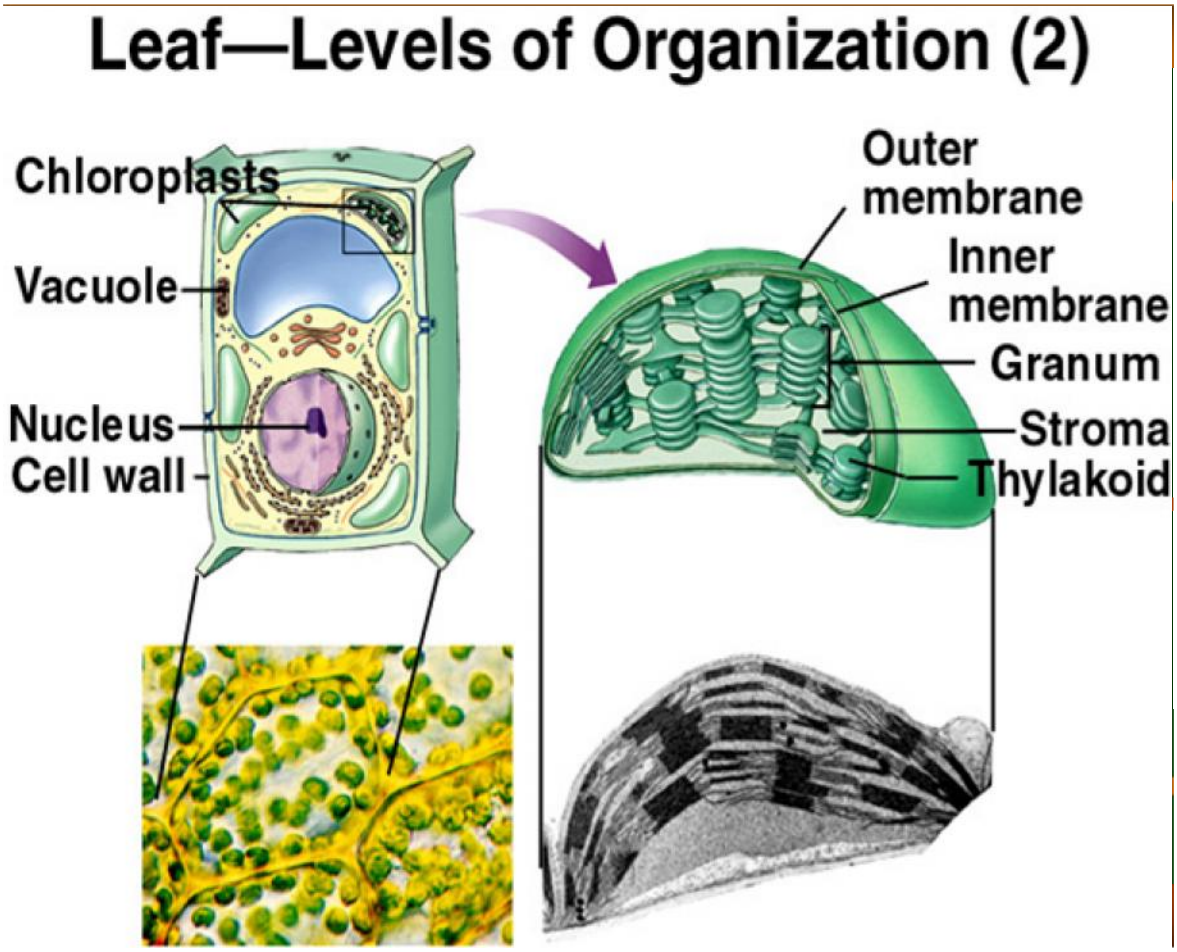
**Chloroplasts** belong to another group of double membrane–enclosed organelles called plastids. Chloroplast membranes are rich in glycosylglycerides. Chloroplast membranes contain chlorophyll and its associated proteins and are the sites of photosynthesis. In addition to their inner and outer envelope membranes, chloroplasts possess a third system of membranes called thylakoids. A stack of thylakoids forms a granum (plural grana) (Figure 1.16B). Proteins and pigments (chlorophylls and carotenoids) that function in the photochemical events of photosynthesis are embedded in the thylakoid membrane. The fluid compartment surrounding the thylakoids, called the stroma, is analogous to the matrix of the mitochondrion. Adjacent grana are connected by unstacked membranes called stroma lamellae (singular lamella). The different components of the photosynthetic apparatus are localized in different areas of the grana and the stroma lamellae. The ATP synthases of the chloroplast are located on the thylakoid membranes . During photosynthesis, light-driven electron transfer reactions result in a proton gradient across the thylakoid membrane. As in the mitochondria, ATP is synthesized when the proton gradient is dissipated via the ATP synthase. Plastids that contain high concentrations of carotenoid pigments rather than chlorophyll are called chromoplasts. They are one of the causes of the yellow, orange, or red colors of many fruits and flowers, as well as of autumn leaves

(Figure 1.17). Nonpigmented plastids are called leucoplasts. The most important type of leucoplast is the amyloplast, a starchstoring plastid. Amyloplasts are abundant in storage tissues of the shoot and root, and in seeds. Specialized amyloplasts in the root cap also serve as gravity sensors that direct root growth downward into the soil (see Chapter 19). Mitochondria and Chloroplasts Are Semiautonomous Organelles Both mitochondria and chloroplasts contain their own DNA and protein-synthesizing machinery (ribosomes, transfer RNAs, and other components) and are believed to have evolved from endosymbiotic bacteria. Both plastids and mitochondria divide by fission, and mitochondria can also so undergo extensive fusion to form elongated structures or networks

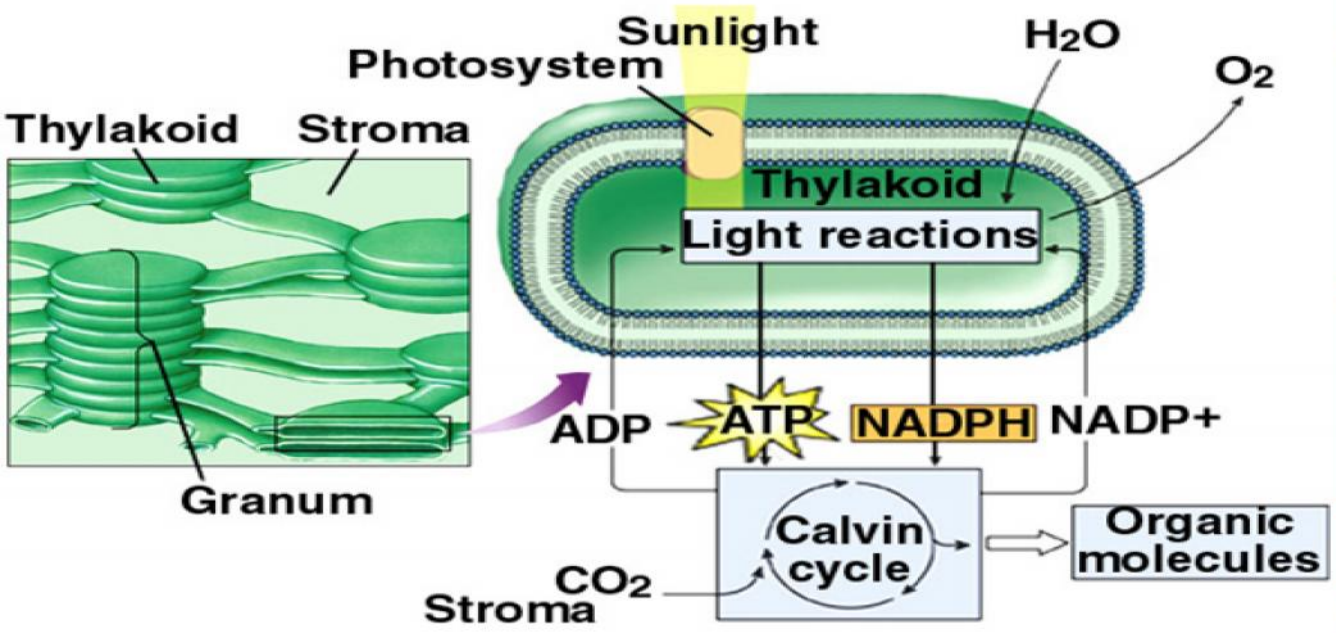


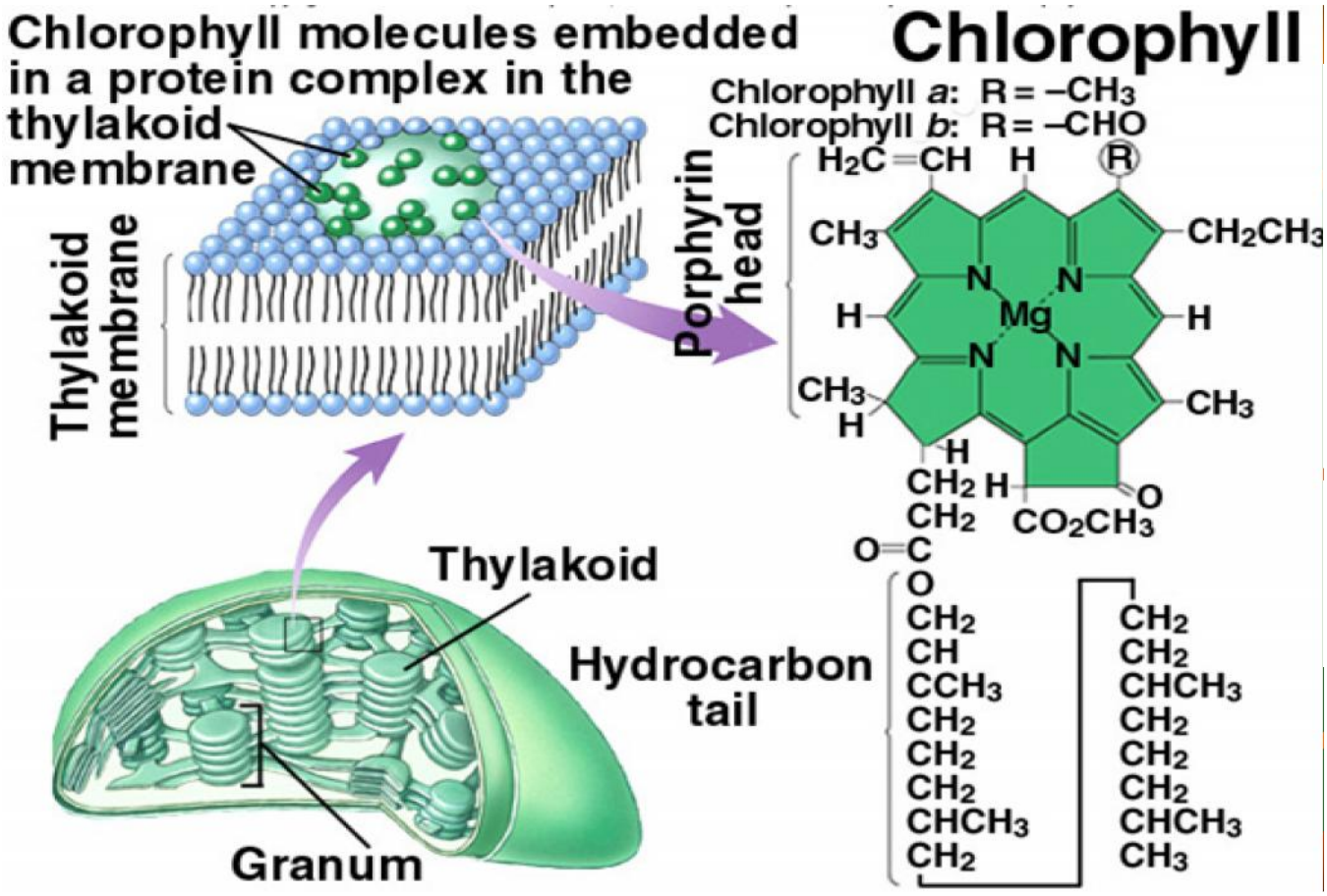



**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.



# Leaf—Levels of Organization (3)






**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

**Plant Cells Contain Microtubules, Microfilaments, and Intermediate Filaments** :

Three types of cytoskeletal elements have been demonstrated in plant cells: microtubules, microfilaments, and intermediate filament–like structures. Each type is filamentous, having a fixed diameter and a variable length, up to many micrometers. Microtubules and microfilaments are macromolecular assemblies of globular proteins. Microtubules are hollow cylinders with an outer diameter of 25 nm; they are composed of polymers of the protein tubulin. The tubulin monomer of microtubules is a heterodimer composed of two similar polypeptide chains (α- and β-tubulin), each having an apparent molecular mass of 55,000 daltons (Figure 1.21A). A single microtubule consists of hundreds of thousands of tubulin monomers arranged in 13 columns called protofilaments. Microfilaments are solid, with a diameter of 7 nm; they are composed of a special form of the protein found in muscle: globular actin, or G-actin. Each actin molecule is composed of a single polypeptide with a molecular mass of approximately 42,000 daltons. A microfilament consists of two chains of polymerized actin subunits that intertwine in a helical fashion (Figure 1.21B). Intermediate filaments are a diverse group of helically wound fibrous elements, 10 nm in diameter. Intermediate filaments are composed of linear polypeptide

monomers of various types. In animal cells, for example, the nuclear lamins are composed of a specific polypeptide monomer, while the keratins, a type of intermediate filament found in the cytoplasm, are composed of a different polypeptide monomer. In animal intermediate filaments, pairs of parallel monomers (i.e., aligned with their —NH2 groups at the same ends) are helically wound around each other in a coiled coil. Two coiled-coil dimers then align in an antiparallel fashion (i.e., with their —NH2 groups at opposite ends) to form a tetrameric unit. The tetrameric units then assemble into the final intermediate filament (Figure 1.22). Although nuclear lamins appear to be present in plant cells, there is as yet no convincing evidence for plant keratin intermediate filaments in the cytosol. As noted earlier, integral proteins cross-link the plasma membrane of plant cells to the rigid cell wall. Such connections to the wall undoubtedly stabilize the protoplast and help maintain cell shape. The plant cell wall thus serves as a kind of cellular exoskeleton, perhaps obviating the need for keratin-type intermediate filaments for structural support. Microtubules and Microfilaments Can Assemble and Disassemble In the cell, actin and tubulin monomers exist as pools of free proteins that are in dynamic equilibrium with the polymerized forms. Polymerization requires energy: ATP is required for microfilament polymerization, GTP (guanosine triphosphate) for microtubule polymerization. The attachments between subunits in the polymer are noncovalent, but they are strong enough to render the structure stable under cellular conditions. Both microtubules and microfilaments are polarized; that is, the two ends are different. In microtubules, the polarity arises from the polarity of the α- and β-tubulin heterodimer; in microfilaments, the polarity arises from the polarity of the actin monomer itself. The opposite ends of microtubules and microfilaments are termed plus and minus, and polymerization is more rapid at the positive end.

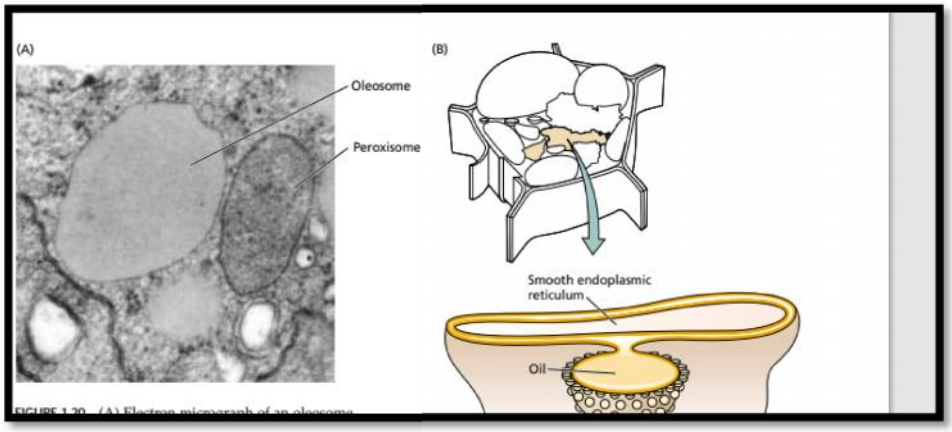



**Fig.** 1.5. Electron micrograph of an oleosome beside a peroxisome. (B) Diagram showing the formation of oleosomes by the synthesis and deposition of oil within the phospholipid bilayer of the ER. After budding off from the ER, the oleosome is surrounded by a phospholipid monolayer containing the protein oleosin. (A from Huang 1987; B after Buchanan et al. 2000.

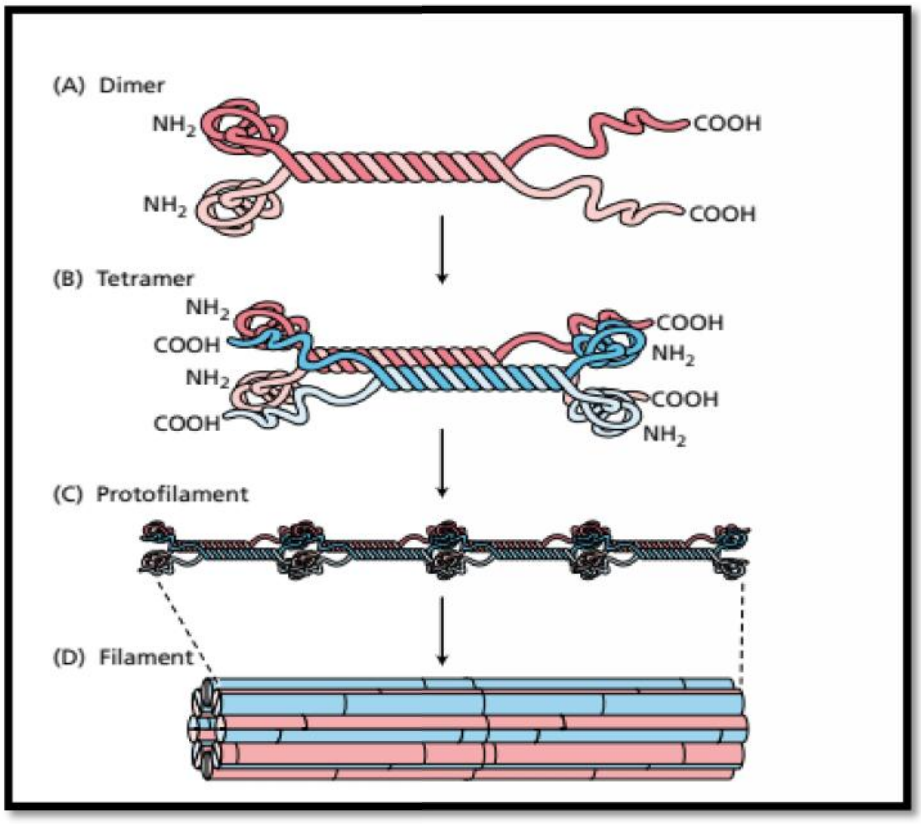
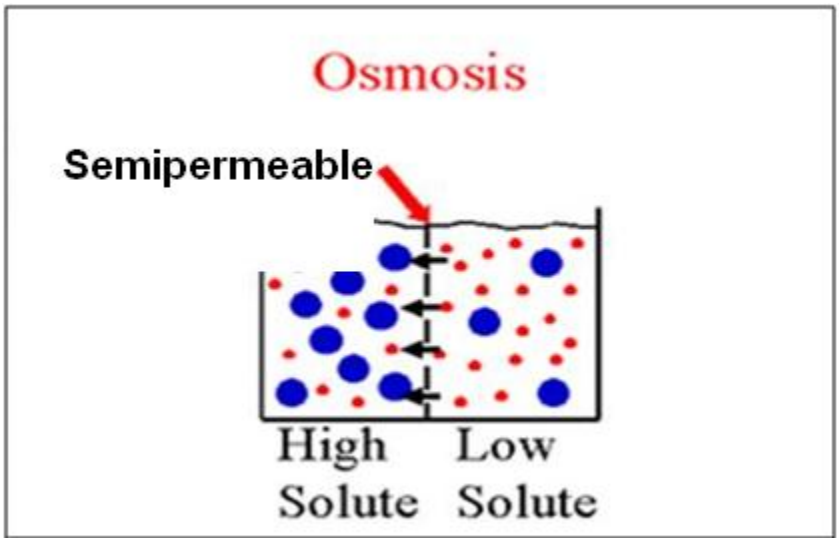



**FIGURE** 1.6. The current model for the assembly of intermediate filaments from protein monomers. (A) Coiled-coil dimer in parallel orientation (i.e., with amino and carboxyl termini at the same ends). (B) A tetramer of two dimers. Note that the dimers are arranged in an antiparallel fashion, and that one is slightly offset from the other. (C) Two tetramers. (D) Tetramers packed together to form the 10 nm intermediate filament. (After Alberts et al. 2002.)

## 3. LECTURE NOTES

### Diffusion of water: Diffusion, osmosis and imbibition, plasmolysis measurements of water status in plants, water potential and its components.

Although the two forms of diffusion are similar, osmosis and imbibition are unique phenomena and play an important role in plant development. Osmosis may be considered as special type of diffusion characterized by the movement of water through a differentially permeable membrane. Imbibition is a special type of diffusion in which an adsorbent is involved.




## Osmotic potential and Pressure

The osmotic pressure can be demonstrated and measured by a simple apparatus termed as **osmometer** in which two compartments are separated by differentially permeable membrane .The pressure that a solution would have to build up to increase its chemical potential to that of pure water is termed **Osmotic pressure**. Osmotic pressure for a solution is the pressure (energy that is depleted by the solution process) that would have to be applied to stop the diffusion of pure water into the solution under ideal osmotic conditions. Thus osmotic pressure is actually a potential and is not usually reached or measured in plant cells. It is a measure of the absence of energy to do work or capacity to flow in an ideal osmotic situation.

For example a 1 molal solution of an undissociated substance in a beaker at $0^0$C may be designated as having an osmotic pressure of 22.4 atm or 22.7 bars. The solution is not exerting pressure but has less energy than pure water, the amount of which depends upon the amount of solute in a given volume of water. The energy lost during the solution process may be restored by the application of external energy via the piston in osmometer or the influx of water into a confined system such as a plant cell. Thus plant scientists use the term osmotic potential, usually designated by $\Psi$ , to describe the absence of energy in a solution, due to the amount of solvent-solute interactions, as compared with pure water under ideal osmotic conditions. In returning to Gibbs free energy relationships, use of a negative sign is justified for osmotic potential value because the solution process is characterized by:

$G_2 - G_1 = -\Delta G$

Where:

$G_2$ = situation after solvation

$G_1$ = situation before solvation

Thus a molal sucrose solution at 0 $^0$C has an osmotic potential of -22.4 atm or – 22.7 bars. These values were obtained by Van't Hoff, who applied the gas law equation to solutions and calculated osmotic pressures of solutions accordingly:

$$II = \frac{N}{V} x\ RT \text{ or } II = CRT$$

Where: II= osmotic potential, N = numbers of moles, V= volume in liters, R= gas constant, T= absolute temperature,

$$C = \frac{N}{V} = \text{Concentration}$$

The negative sign is inserted to denote osmotic potential that it characterizes a solution in several ways. It indicates the maximum pressure ( osmotic) that might develop if the solution were allowed to come to equilibrium with pure water in an ideal osmotic system, and it is proportionately related to the amount of solutes in a solution

and to the decrease in chemical potential ( total free energy ) due to solute- solvent interactions.

## Turgor pressure

A rigid, relatively inelastic structure, the cell wall, encloses the plant cell and its differentially permeable plasmalemma. This unique feature of the plant cell allows it to survive in a wide range of osmotic concentrations. On the other hand, the animal cells can live only in solutions in which the osmotic concentrations are identical (isotonic) or nearly identical to that of the cell contents.

The plant cell, when placed in pure water, swells but does not burst due to negative osmotic of the vacuole solution (cell sap), water will move into the cell and will cause the plasmalemma to be pressed against the cell wall. The actual pressure that develops, that is the pressure responsible for pushing the membrane against the cell wall is **Turgor pressure (or hydrostatic pressure)** which can also be defined as pressure exerted by protoplast over cell wall which pushes the plasma membrane against the rigid cell wall and provides a force for cell expansion.

The cell wall, being rigid, exerts an equal and opposite pressure called **wall pressure** which can also be defined as pressure exerted by the cell wall over protoplast. The first, easily observed sign of a water deficit in a plant is a decrease in the turgor of its leaf cells giving the leaves wilted appearance.

**Diffusion pressure deficit –** Wall pressure tends to force the water out of cell and acting against the osmotic entry of water in the cell. Now absorption of water takes place which depends upon difference between osmotic pressure and wall pressure. During this time wall pressure equals turgor pressure. This difference is called **diffusion pressure deficit** which is the difference between osmotic pressure and turgor pressure and can be expressed as follows:

DPD = OP-TP (WP), TP= OP-DPD, OP=TP+DPD

## Water potential

The chemical potential is the free energy per mole of any substance in a chemical system. Generally chemical potential of water is referred as **Water potential (Ψw).** When water potential is expressed it is expressed as the difference between the chemical potential of water at any point in a system (μw) and that of pure water under standard conditions ($\mu w^{o}$) with the expression:

$$\Psi w = \mu w - \mu w^{o} = RT \ln \frac{e}{e^{o}}$$

Where, R is the gas constant (erg/mol /degree), T is the absolute temperature ($^{o}K$),e the vapour pressure of the solution in the system at temperature T and the vapour pressure of pure water at the same temperature. The expression RT in ($e / e^{o}$) is zero which indicates that pure water has a potential of zero. In a biological system ($e / e^{o}$) is less than zero making in ($e / e^{o}$) negative. Pure water is defined as having a potential of zero, any dilution of water with a solute establishes a potential that is less than that of pure water and expressed as a negative number. Both water potentials and chemical potentials can be expressed in energy units. It is more convenient to express water potentials in pressure units (atmosphere, bars, megapascal) which can be obtained by dividing water potential by partial molar volume of water (Vw):

$$\frac{\mu_w - \mu_w^{o}}{V_w} = \frac{RT \ln \frac{e}{e^{o}}}{V_w}$$

The units of the above equation are:

$$\frac{\text{Erg/mole}}{\text{Cm}^2/\text{ mole}} = \frac{\text{erg}}{\text{cm}^3} = \text{dyne/cm}^2$$

1bar = 0.987 atm. $=10^{6}$ dynes/$cm^{2,}$

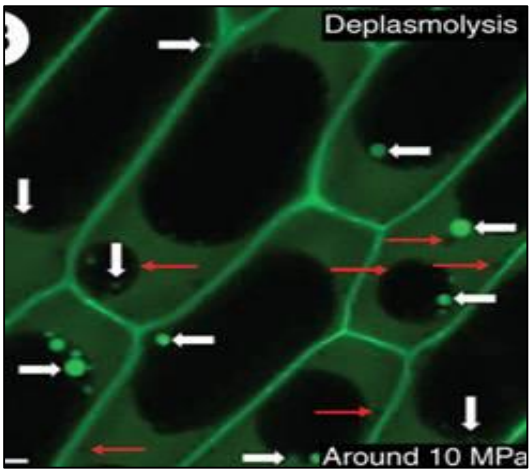
10bars = 1megapascal (mpa)

If some substance such as sugar is dissolved in pure water contained in a beaker, the resulting solution has an osmotic potential that is lower (more negative) than that of pure water. Since this is an unconfined solution (not under the pressure of a piston or cell wall, the turgor pressure is zero. An increase in solute decreases the free energy and will produce more negative osmotic hence water potential.

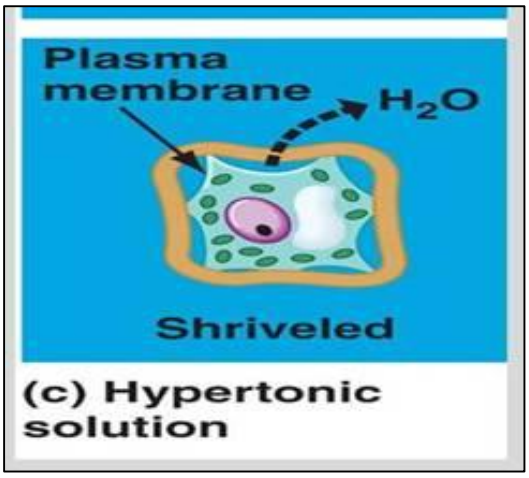
**Plasmolysis:–** Shrinking of the protoplasm of a cell placed in an hypertonic (low osmotic potential) solution, away from the cell wall, due to loss of water. If we place a living plant cell in a solution with an osmotic potential to that of its own cell sap **(an isotonic solution)**, the appearance of the cell remains normal in every respect. However, if the water potential of the surrounding solution is less negative than that of cell sap (hypotonic) or more negative than that of cell sap **(hypertonic)**, we can easily observe several changes in cell structure. If we immerse epidermal tissue from the leaves of Rhoeo or Zebrina in a hypertonic solution of sucrose, we can observe the plasmalemma pulling away from the cell wall which is referred to as **plasmolysis**. In the first case the water inside the cell has a greater free energy and thus a greater tendency to flow outward. Second, the cell and vacuolar membranes are practically impermeable to sucrose but readily preamble to water. Third, the cell wall will allow the free passage of both sucrose and water. Thus there will be a net movement of water out of the cell vacuole and into the external solution, water will move from a region of less negative (high) to a region of more negative (low) water potential. This movement of water results in a loss of turgor, a shrinking of the vacuole, and pulling away of the cell membrane from the cell wall. **Incipient plasmolysis** is the initial pulling away of the membrane from the cell wall. At this point the turgor pressure is zero. If the process continues, there will be a tendency for the cell wall to be pulled towards the cytoplasm because of cohesive and adhesive properties of water between the cell wall and plasmlemma. This cell is then said to be under tension , and the turgor pressure becomes negative. Eventually, the forces exerted by the retracting plasmalemma will

become greater than those between the water molecules of the cell wall. Complete plasmolysis follows, with the plasmalemma being pulled entirely away from the wall.

**Hypotonic**




**Deplasmolysis**




**Plasmolysis**

## Deplasmolysis

Regaining of original state by plant cell or tissue as a result of water absorption**.** Plasmolysed cells can be deplasmolysed, if a cell that has been plasmolysed is placed in a hypotonic solution, it will regain its turgidity. A different condition develops if a living plant cell is placed in a solution that is hypotonic to the cell sap. In this situation, water moving from a region of less negative water potential ( the external solution) to one of more negative water potential ( the cell sap )will enter the cell and cause it to become more rigid. Since cell wall is elastic to some degree, the cell volume will increase slightly. The turgor pressure of the cell will also increase. Because the increase in the volume of the cell in a hypotonic solution is generally very small, it is difficult to observe any difference in appearance between a plant cell in an isotonic solution and a plant cell in a hypotonic solution.

## Osmotic potential measurements

The boiling point of an aqueous solution is higher than that of pure water, the vapour pressure of the water in a solution is lower than that of pure water, and a solution freezes at a lower temperature **(freezing point depression)** than pure water. These factors, called the colligative properties of solutions, are interrelated, and the extent to which each factor is affected is directly proportional to the number of dissolved particles (molecules or ions) present. Therefore, a measure of any one of these factors is an indirect measure of the osmotic potential because it is also one of the colligative properties of the solutions. Generally, we do not use boiling point elevation to measure the osmotic potential of the cell sap. However, we can measure the vapour pressure depression and freezing point depression of expressed plant juices with a considerable degree of accuracy. For example, the theoretical freezing point depression of a 1 molal solution composed of nonionised solute has a freezing point depression of $-1.86^0$ C and a theoretical osmotic potential of − 22.7 bars(−22.4 atm.).We can easily arrive at an equation relating these two factors, freezing point depression and osmotic potential and we can use this equation to determine the osmotic potential of a solution of unknown concentrations.

Therefore,

$$\Psi s = \frac{-22.7x\,\Delta fp}{-1.86}$$

In this equation, Δ stands for the observed freezing point depression of the unknown solution. If, for example, some plant juice is expressed and found to have a freezing point depression of 1.395, the osmotic potential of this solution would be:

$$\Psi s = \frac{-22.7 \times -1.395}{1.86} = 17.02\ 5\text{bars}$$

The determination of a solution's osmotic potential by determination of its freezing point is called **Cryoscopy** and the technique is referred to as **Cryoscopic** method.

Another method of determining osmotic potential makes use of plasmolytic phenomenon. A graded series of solutions, covering a certain range of osmotic potentials (water potentials) is prepared. The solutions which are usually of sucrose's, are prepared so as to provide a graded series in which some of the solutions are hypotonic and others are hypertonic to the cells to be treated. Strips of plant tissue, preferably tissue containing anthocyanins, are placed in different solutions and after a time of around 30 minutes are placed under the microscope. Examination of the strips of tissue from the different solutions will show some in which all of the cells are turgid, some in which nearly all of the cells are plasmolysed, and some in which 50 percent of the cells are just beginning to show signs of plasmolysis ( incipient plasmolysis) .At incipient plasmolysis , the turgor pressure of the cell is zero, and the osmotic potential of the cell contents is equal to the water potential of the cell and to the water and the osmotic potentials of the external solution.

## Water potential measurements

## Volume method

The volume method of measuring water potential is based on changes in linear dimensions (length) of a tissue when it is placed in solutions of different osmotic potentials. When solutions are placed in a beaker, there is no turgor pressure and solutions are unconfined and at this moment $\Psi w = \Psi s$.This situation is not true for plant cells. Strips of root, fruit or leaf tissue, 3 to 4 cm long and of the same width, are measured and placed in the series of different concentrations of sucrose solutions for about 1 hour. The tissues are removed and remeasured. The change in length is then plotted against the known osmotic potentials of the solution. The water potential of the solution $\Psi w = \Psi s.+ 0$ in which the tissue does not change in length is the same as the water potential ( $\Psi w = \Psi s.+ ?$ ) of the tissue.

## Gravimetric method

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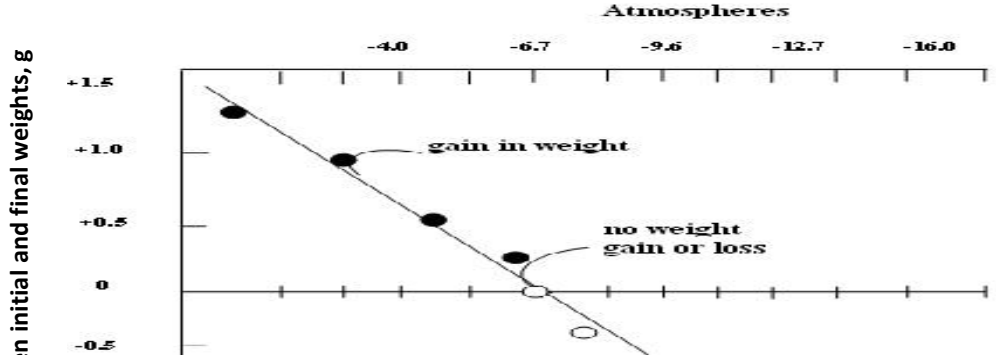


This method involves the placement of preweighed plant tissue into a graded series of solutions of sucrose or other **osmoticum (osmoticlly active solute)** at known osmotic potential.

A representative sampling of tissue is incubated for predetermined time in the solutions, removed and reweighed. The weight gain or loss is plotted against the water potential of each solution. When the points are connected, the intercept at the abscissa (through zero) represents the water potential of the tissue, with the zero weight gain or loss. The water potential of the solution corresponding to the intercept point is equal to that of the tissue.

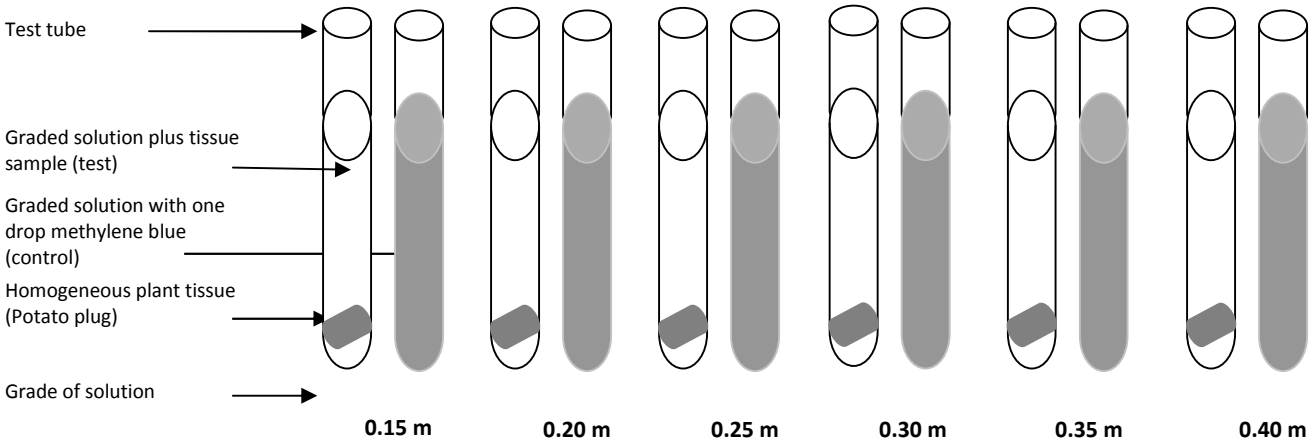
## Chardakov's or Falling drop method

Two graded series of sucrose solutions (ranging from 0.15 to 0.50 molal in increments of 0.5 molality) are placed in test tubes set up in duplicate. Homogenous plant tissue is placed into each test tube of one of the series (test series). Only one drop of methylene blue is mixed into each solution of the second series (control series). Plant tissue is not added to the control series and the dye does not appreciably change the osmotic potentials.

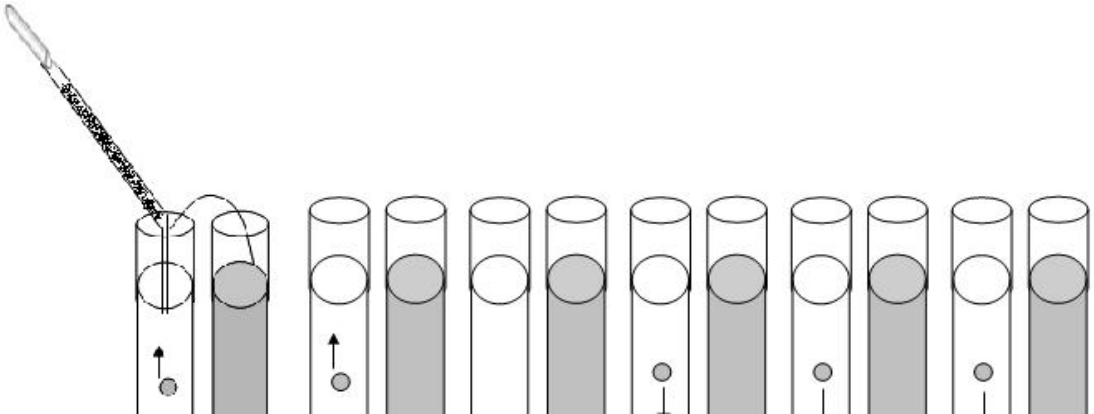
After the tissue has incubated for 15 to 30 minutes, it is removed from each tube. The actual time of incubation can be just long enough for osmosis to proceed and change the concentration of each solution in the test series, the attainment of equilibrium is not necessary. After the tube is removed, a small drop of the respective control series solution is introduced below the surface of its corresponding test solution.

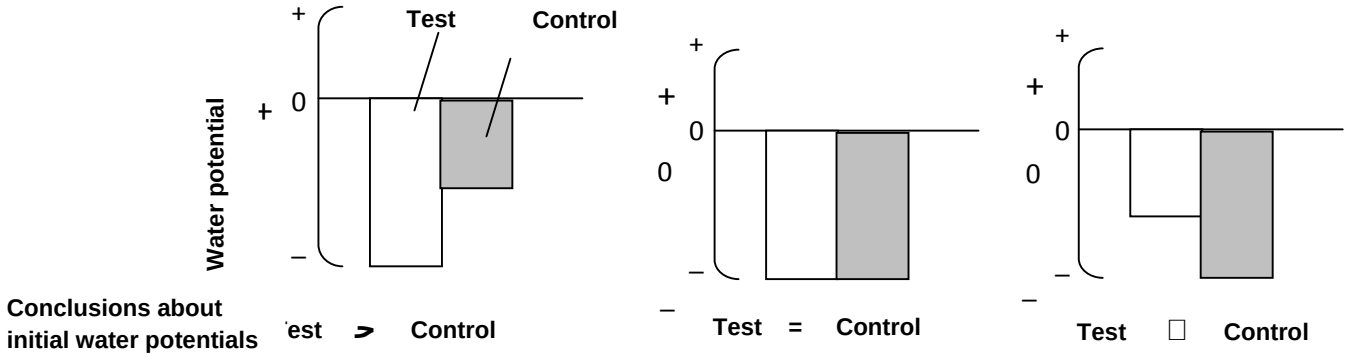
If the drop rises in the test solution, it means that the drop is lighter and that the tissue inoculation solution is more concentrated and this indicates that water from the solution entered the tissue. If drop falls, it means that the test solution is lighter which indicates that water has left the tissue and diluted the solution. In the latter instance, the water potential of the solution initially is more negative than that of the tissue. Accordingly, if the density of the drop from the methylene blue solution is the same as that of the test solution, the drop will diffuse into the solution uniformly. At this point (called the null point), the water potential of the tissue and solution is equal.

It is also possible to determine the solution changes with a refrectometer (refrectometer method) instead of falling drop. The refrectometer is used to measure directly the concentration changes that take place in the tissue incubation solutions. No change in concentration indicates that the solutions have the same water potential as that of the tissue cells. This method does not require the methylene blue dye and experimental error due to technique is minimized.




Step 2 – Incubate series for 15 to 30 minutes
Step 3 – Remove tissue and introduce drop of control solution into test solution






## Vapour Pressure

**(Thermocouple Psychrometer Method)**

The vapour pressure is based on the fact that tissue will not gain or lose water to the atmosphere when the vapour pressure of air corresponds to the water potential of the tissue. The most extensively used apparatus is constructed for measurements of humidity inside a closed chamber containing two thermocouple junctions. One remains at the temperature of the air in the chamber, the other cools rapidly when a weak current is passed through the two junctions. Moisture from the air in the chamber will eventually condense on the cooling thermocouple. The drop of moisture then acts as a wet bulb. The water potential of the air in the chamber is equal to the difference between the temperature of the wet bulb and that of the dry thermocouple.

## Pressure Bomb

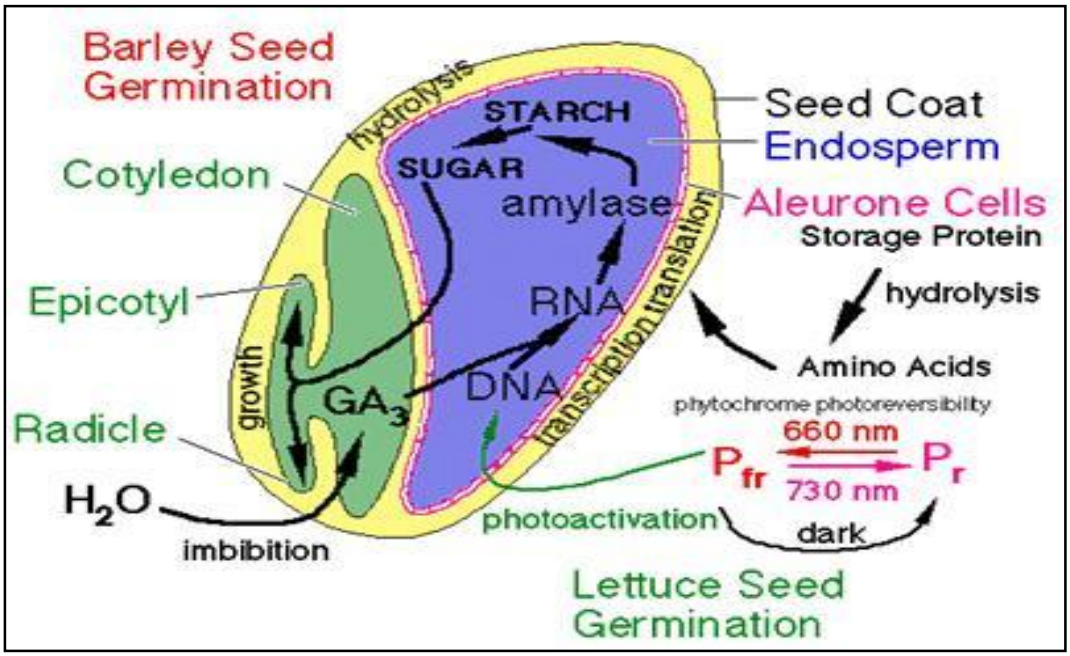
Pressure bomb is the device that is used to determine the plant moisture stress and the water potential of a leafy shoot and is based on the assumption that the water column in a plant is almost always under tension because of the pull exerted by the osmotic influences (water potential) of the living cells of the leaves. If the tension is high, the water potential of the leaf cells is very negative. When a

stem is cut, the water column is disrupted, and because the water column is under tension, it will recede back into the stem towards the leaves. The shoot is placed in the chamber, with the cut end protruding through an air tight hole. Pressure is increased within the chamber, and the water column within the twig is forced back to the cut surface. The pressure in the chamber is then carefully recorded.

The pressure required to force the water to appear at the cut surface is equal to the tension (but with the opposite sign) of the water column at the time the shoot was cut. If low pressure is sufficient to force water to appear at the cut surface of the shoot, the living cells mainly of the leaves have slightly negative water potentials, with the shoot being under relatively low moisture stress. But high pressure is required to force water to the cut surface the moisture stress(tension) is relatively high due to very negative water potentials of the leaf cells.

## Imbibition

It is the adsorption of water by the hydrophilic colloids of plant material. As with osmosis, imbibition may be considered a special type of diffusion since the net movement of water is along a diffusion gradient. In this case an adsorbent is involved. If dry plant material is placed in water, a noticeable swelling takes place and sometimes amounts to a considerable increase in volume. Tremendous pressures can develop if an



adsorbent is confined and then allowed to imbibe water. For example, dry wooden stakes, driven into a small crack in a rock and then soaked, can develop enough pressure to split the rock.

## Conditions necessary for imbibitions

Two conditions are necessary for imbibition to occur 1. A water potential gradient must exit between the surface of the adsorbent and the liquid imbibed. 2. A certain affinity must exist between components of the adsorbent and the imbibed substance. Very negative water potentials exist in dry plant materials. For example, water potentials of −900 bars have been recorded in some dry seeds. Therefore, when this material is placed in pure water, a steep water potential gradient is established, and water moved rapidly to the surface of the adsorbent. As water continues to be adsorbed, the water potential becomes less negative till it equals to that of external water. At this point an equilibrium is established, imbibition ceases, and water moves to and from the adsorbent in equal quantity.

An adsorbent does not necessarily imbibe all kinds of liquids. For example, dry plant materials immersed in ether do not swell appreciably. On the other hand rubber will swell when submersed in ether, but rubber will not imbibe water. The obvious implication is that certain attractive forces must exist between components of imbibant and the imbibed substance.

A considerable amount of colloidal material is present in both living and dead plant cells. Proteins and polypeptides are hydrophilic colloids having strong attraction for water. The plant cells possess a considerable amount of carbohydrates in the form of cellulose and starch to which the water is strongly attracted. The adsorption of water to the surfaces of these hydrophilic colloids is of major importance to the imbibition process. Seeds which are high in colloidal material are very good adsorbents. Water is brought into the germinating seeds largely through the process of imbibition.The water potential of a biological system is made more negative by the presence of these adsorptive or water binding materials. To these materials or to the forces they generate the term matric potential $\Psi m$ is applied. In recent years the term matric potential has

replaced the old term imbibition pressure and is somewhat analogous to osmotic potential.

## Matric potential

The term Matric potential is analogous to osmotic potential in that it represents the potential maximum pressure that an adsorbent will develop if submersed in water. The actual pressure that develops when water is imbibed may be thought of as turgor pressure (pressure potential). Accordingly the following equation can be presented:

$\Psi w = \Psi m + \Psi p$

The equation is similar to the one used for osmotic systems, where osmotic potential is equal to osmotic potential plus turgor pressure. The matric potential is always negative. No turgor pressure develops in an unconfined adsorbent. Accordingly the above equation under these conditions simplifies to:

$\Psi w = \Psi m.$

The matric potential of air dried seeds such as cocklebur may approach − 1000 bars (Schull 1916,1920). If the seeds are immersed in pure water, the water potential of the very small amount of water in the dry seeds would be nearly − 1000 bars. After imbibition ceases, the water potential of the external or internal water is zero. If the seeds are submersed in water having water potential of −500 bars in a solution of NaCl with an osmotic potential −50 bars (water potentials equals−50 bars), the water potential of the seed water at equilibrium will be −50 bars. As in osmotic systems, the water potentials tend to equilibrate.

## Factors affecting rate and extent of imbibitions

The rate and extent of imbibition is affected primarily by the temperature and by the osmotic potential of the substance to be imbibed. Temperature does not affect the amount of water taken up by the adsorbent, but it does have a definite effect on the rate of imbibition. An increase in temperature causes an increase in the rate of imbibition. Both the amount of water imbibed and the rate of imbibition are affected by the osmotic

potential of the substance to be imbibed. The addition of solute to pure water causes a more negative water potential. This addition has the effect of altering the water potential gradient between the solution water and the adsorbent. The water potential gradient is less steep than it would be if the same adsorbent was submersed in pure water. Similarly, a decrease in the water potential gradient will bring about a decrease in the rate at which water is imbibed and thus the amount of water taken up.

## Volume and energy changes

The volume of an adsorbent increases as a result of imbibition. However, the total volume of the system (the volume of the water in which the adsorbent is submersed plus the volume of the adsorbent) is always less after imbibition than before imbibition starts. This can be easily demonstrated by placing air dried seeds in a graduated cylinder containing water, reading the initial volume and comparing it with the volume of the system after imbibition ceases. This reason for the difference in volume is the water molecules adsorbed to the surfaces of colloidal material present in the adsorbent are held relatively tightly. As a result, they are packed close together and resulted in decrease in the volume of the system. Due to tight adsorption of water molecules, some of the kinetic energy possessed by these molecules is lost which can be shown in the system as heat. Therefore, there is always an increase in temperature as a result of imbibition.

## 5. LECTURE NOTES

**Absorption of water: water absorbing system of plant, kinds of soil water in relation to water absorption.**

### Terms contacted with soil water

**Soil water:** Soil is a great reservoir of water for plants.

**Run away water:** After a heavy rainfall or excess irrigation some part of water drains away along slopes which is not available for plant growth.

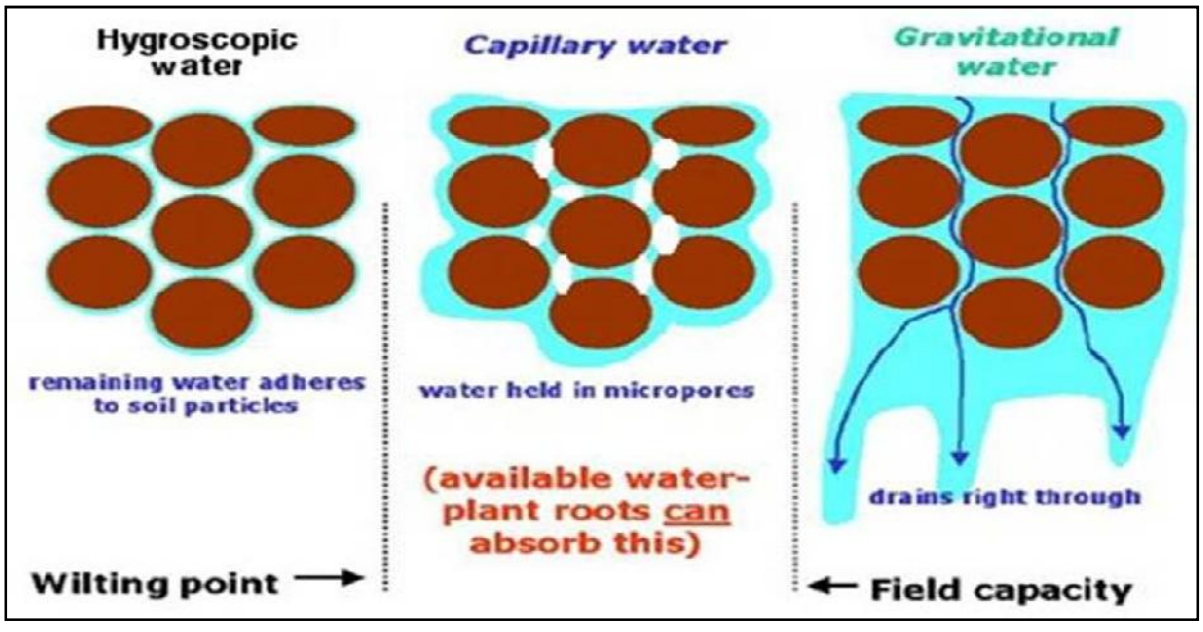
**Gravitational water:** Some part of water percolates downward through larger pores between soil particles under influence of gravitational force till it reaches the water table, not available for plant growth.

**Hygroscopic water:** Water adsorbed on the surface of soil colloids in the form of tightly held thin film. Not available for plant growth.

**Capillary water:** Water fills the spaces between noncolloidal smaller soil particles and forms films around them, available for plant growth.

**Chemically combined water:** Water bounds to soil minerals by strong chemical bonds, not available for plants.

### Field capacity or water holding capacity



Much of the rain water is retained by soil particles against the force of gravity and makes the soil wet. The amount of water which soil retains after the excess amount of water is removed is also called field capacity.

## Water table

At some depth in soil all the pore spaces are filled with water. If a hole is bored in the soil water will appear at this point.

## Water use efficiency

Ratio between the gain of (above-ground) biomass in growth or $CO_2$ in photosynthesis and transpirational water loss.

## Wilting coefficient or wilting point

Amount of moisture left in soil after a plant has wilted. This is expressed as a percentage of dry weight of soil. It is lowest for sandy soil, high for loam soils and still higher for clayey soils. Osmotic pressure at permanent wilting becomes 15 atmosphere.

## Temporary or transient wilting

Despite of sufficient moisture in soil plants may exhibit sign of willing. This happens generally in a afternoon of a hot summer day when transpiration exceeds absorption but plants recover again at night automatically when transpiration is reduced and absorption is still continued. This type of wilting is associated with the diurnal fluctuation of water content.

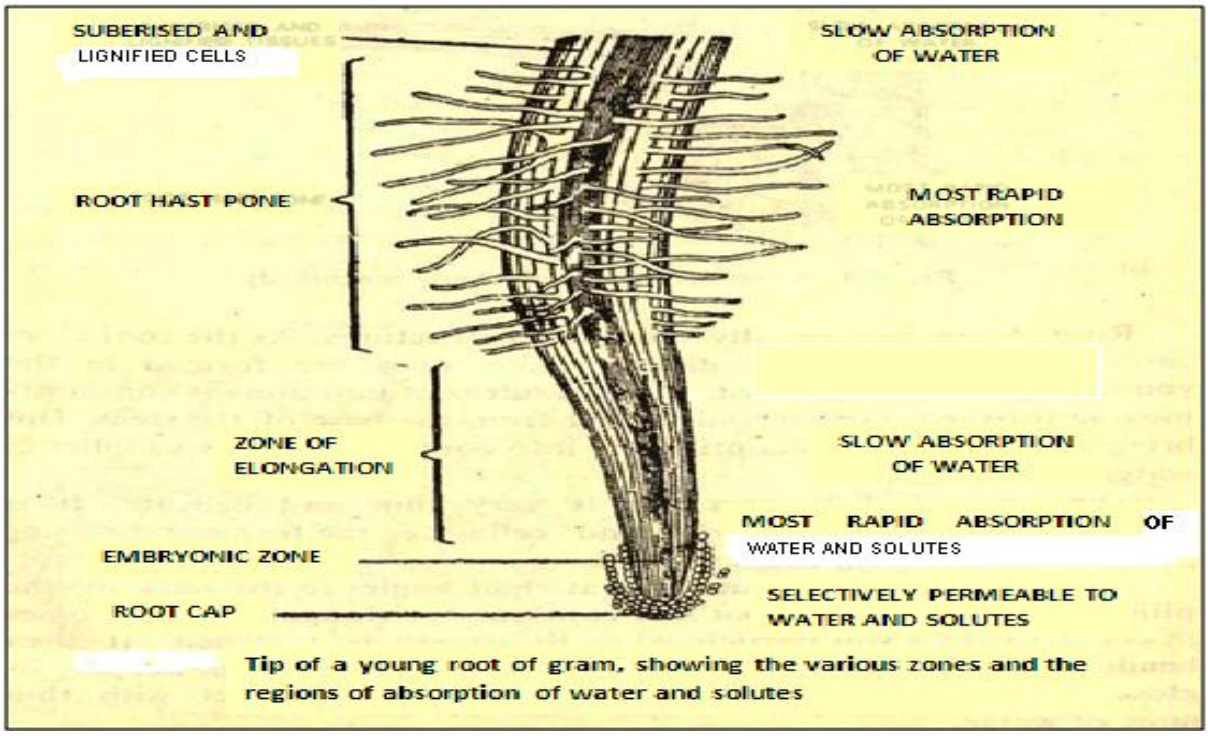
## Permanent wilting

Wilting caused by an actual lack of water in soil. A plant will not recover unless water is added to the soil. In most herbaceous plants the leaf water potential is then - 14 to -15 bars.

## Water absorbing system of plant

The main part of the plant which is concerned with water absorption is the root. The depth of roots varies with different species and greatly affected by factors like

water, mineral nutrients, $O_2$ and temperature. Some species are deep feeders possess deep root system, whereas, some are surface feeders associated with shallow root system.




The absorption of water does not take place from entire surface of root. Only younger portions near the tip are active in absorption of water and mineral substances. More number of tips in roots favour higher absorption. Extensively developed root system is associated with higher absorption rates in view of more number of active tips in roots.

At the extreme tip of root, root cap exists which consists of mass of cells forming a protective sheath around growing point. The growing points are about one mm in length and made up of embryonic cells which are rapidly dividing. This region is called embryonic zone. This zone is associated with higher absorption rate. Behind this lies a zone of elongation where growth of root takes place in length. This region favours very slow absorption rate. This is followed by root hair zone which is covered with root hairs. Root hair zone extends from 1 to 4 cm. Behind this region mature part of root lies

where cell walls become lignified and suberized. This zone is associated with slow absorption rate. Most of the water absorption takes place through the root hairs. A root hair is the tubular extension of epidermal cells. As the root elongates the older root hairs die and new one come up in younger parts of roots. As a result the root hair zone is constantly moving forward farther and farther. This brings the root hairs continuously come into contact with new supply of water in the soil.

## Anatomy of xylem tissue

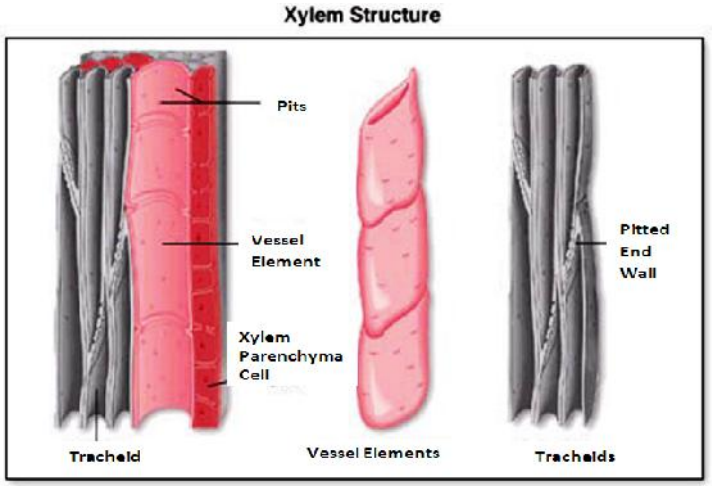
Xylem has been recognized as tissue involved in the water translocation. Several different type of cells, living and nonliving, comprise xylem tissue. Of these, the tracheary elements are most prevalent and through these cells water translocation takes place. Xylem also consists of xylem fibers (scleranchyma) and living parenchyma cells.

## Tracheids and vessels

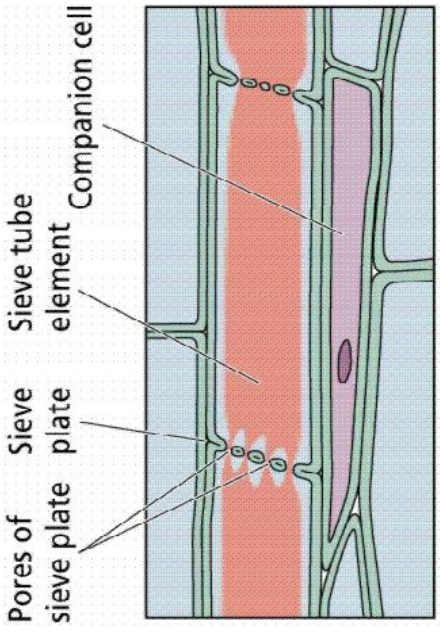
The vessel elements and tracheids are the cells most involved in the water translocation. Both are more or less elongated, have lignified secondary wall and are dead when they are mature and functional. Since vessel elements and tracheids are dead at maturity, there is no interfering protoplast in the lumina of the cells, a situation that allows for the efficient translocation of relatively large amounts of water. Perforated and walls are characteristic of vessel elements but do not occur in tracheids. However, tracheids are well supplied with bordered pits. In the more developed vessel elements, the end walls may be entirely missing leaving nothing to obstruct the passage of water through the cell. If large number of vessel elements are taken and stacked them end to end, we would have a long tube like structure. These structures formed from a series of vessel elements attached to one another by their end walls is called vessel or xylem duct. The vessels of the xylem tissue form a network of ducts that extends to all areas of the plant and gives all cells an easily accessible supply of water. This network is important not only for the maintenance of turgor but also for the translocation of other substances that may be carried from cell to cell by the moving water( e.g. essential mineral elements). In angiosperms the vessel system is the principal pathway by which

the water is translocated. However, vessels are not present in conifers, and in this group tracheids form the principal pathway for water translocation. Tracheids are long spindle shaped cells with sharply inclined end walls which overlap each other and through mediation of bordered pits provide a continuous pathway for the movement of water. As we might expect, the movement of water in a group of tracheids as compared to the vessel system is probably much less direct and meet with more resistance. Nevertheless, water flow does not seems to be impeded in the taller trees, many of which are conifers and which, therefore devoid of vessels. The spring wood consists of tracheids with large lumina and thin secondary cell walls. On the other hand, the summer wood is characterized by cells with small lumina and very thickened secondary cell walls. The growth of the tracheids is directly related to the seasonal growing conditions, particularly the availability of water.

Although vessels and tracheids are originated in the plant in a vertical direction with respect to their long axis, and water movement is predominantly in this direction, lateral water movement does take place. Numerous pits through which water may pass perforate the side walls of vessel elements and tracheids. Generally, where cells lie alongside each other, pits occur in pairs and are called pit pairs. Thus where pits lie adjacent to each other, water may move from cell to cell laterally. Since pit pairs may occur between two vessel elements, two tracheids, a tracheid and a vessel element , a




Structure of xylem



tracheid or vessel element and living parenchyma cells, and so on , water can be easily distributed throughout all the tissues of a plant.

## Xylem fibres

The xylem fibre is a long, thin, tapering cell with a very thick, lignified cell wall and is dead at maturity. The primary function of the xylem fibre is support, but it is also possible for some water to pass through xylem fibres since they are in association with each other and with the tracheids and vessel elements via pit pairs.

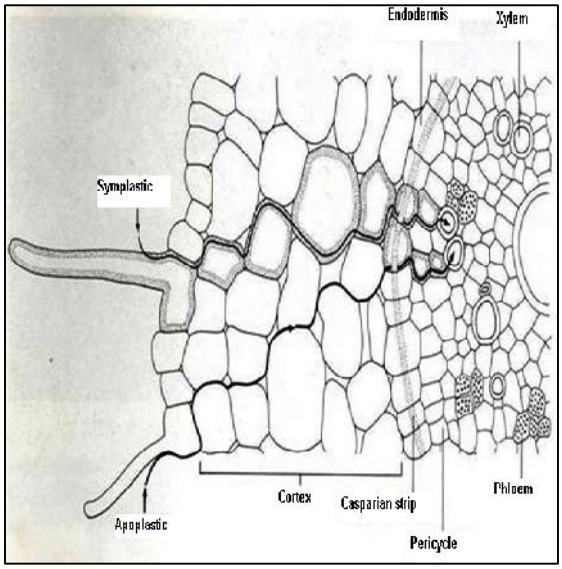
## Xylem parenchyma

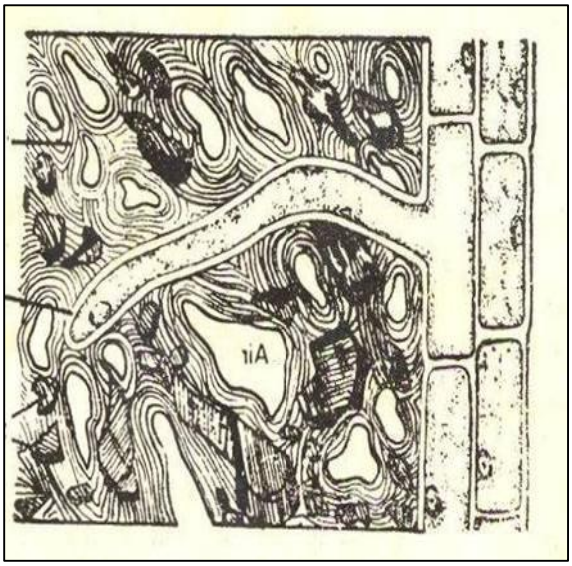
Living parenchyma cells may be found interspread in the conducting cells or as components of xylem rays and are generally referred to as a wood and ray parenchyma, respectively. One obvious function of xylem parenchyma is the storage of food. Starch is accumulated towards the end of growing season and is then depleted during the cambial activity of the following growing season (Goodwin and Mercer 1973). The xylem ray parenchyma generally facilitates the lateral transport of water and nutrients. The living parenchyma cells of the xylem may have a vital role in the translocation of water.

## Mechanism of water absorption

The absorption of water takes place through root hairs which are in contact with water films on soil particles. Inside the root hair is a thin lining of cytoplasm which encloses a large vacuole filled with cell sap. The cell wall of a root hair is a permeable membrane. Its cytoplasmic line is a semipermeable membrane. Cell sap is solution of mineral salts, sugars and organic acids. As a result of it exerts a higher osmotic pressure (3-8 Atm.). The soil solution is relatively dilute and has low osmotic pressure (less than 1 Atm.). Due to this water is absorbed. As the films of capillary water are removed by absorption by root hairs, the films of water from adjacent soil particles are drawn on. These in turn draw water from the particles adjacent to them, by this way water may move from considerable distances to root hairs. The capillary movement of water in soil takes place through cohesive force of water molecules. Root hair cells are in contact

with cortical cells which extends to endodermis. Internal to endodermis is a single layer of parenchymatous cells which lies opposite to protoxylem. This arrangement offers a direct channel for the passage of water to xylem. Endodermis generally consists of thick wall cells with the deposition of lignin, cutin and suberin. Such arrangement makes movement of water across endodermis difficult. In some cases cells of endodermis opposite to xylem do not have such thickenings. These are known as passage cells. These cells allow very easy movement of water across endodermis. As a result of absorption of water from the soil the root hair cell becomes fully turgid, its osmotic pressure falls due to dilution and turgor pressure increases. Due to this its suction pressure will fall below that of adjacent cortical cell b, with the result water will pass from a to b. The diffusion of water into b reduces its suction pressure which falls below that of cortical cell c. With the result water passes from b to c, by this way water reaches to endodermal and pericycle cells which then becomes trurgid. It will exert no suction pressure, hence will give up water to xylem vessels. The walls of xylem vessels are inelastic, so there is no turgor pressure, and the whole of osmotic pressure of xylem sap constitutes its suction pressure. This being higher than the reduced suction pressure of parenchyma (pericycle) cells, water will be drawn into xylem vessels. The force with which water is drawn in from the soil depends upon difference between osmotic pressure of xylem sap and soil solution. The water is pushed into xylem vessels by surrounding cortical cells with certain force. This force is called root pressure . Conditions which hinder water uptake are poor aeration, cold or dry soils, higher concentration of salts in soil, presence of toxic substances etc.



## Absorption of water by aerial parts of the plant

The absorption of water both in liquid and vapour forms occurs to a small extent through aerial parts of most plants. The extent to which this occurs depends on the water potential of leaf cells and the permeability of the cutin layer (Gessner 1956). Roberts, South and Palmiter (1948) found that the cutin layer on the leaves of the McIntosh apple was not continuous but occurred in lamellae parallel to the outer epidermal walls. Interspread with the parallel layers of cutin, they found parallel layers of pectinaceous material of good water absorbing capacity. Not only was this material present with the cutin layer at the surface of the leaf, but it extended vertically to the vein extensions within interior of the leaf. It thus formed a continuous path for water from the surface to the vascular tissue. The permeability of the cutin layer of the apple leaf to water is rather good.

Some investigations believe that water absorbed by the leaves can travel in a negative direction through the plant and can actively diffuse through the roots into the soil. Breazeale, McGeorge and Breazeale (1950, 1953,1951) demonstrated that both tomato and corn plants are capable of moving water absorbed by the leaves back into the soil. The activity only occur along water potential gradients favouring movement in this direction.

## Apoplast and Simplast

Apoplast refers to the dead parts of plant cells viz; all walls, xylem vessels, intercellular spaces, whereas, symplast includes all living parts of plant cells viz; plasmodesmata and elements within cytoplasmic membrane. The terms apoplast and simplast were originally introduced by Munch (1931) in his studies on the flow of water and solution in plants. The terms are convenient for describing the path of absorbed and translocated water and solution. Water may be translocated across the root cortex through a system of interconnecting cell walls and intercellular spaces before reaching the casparian strip of the endodermal walls. Once through the endodermal and pericycle cells, the water wets the xylem cell walls as well. Munch referred to the

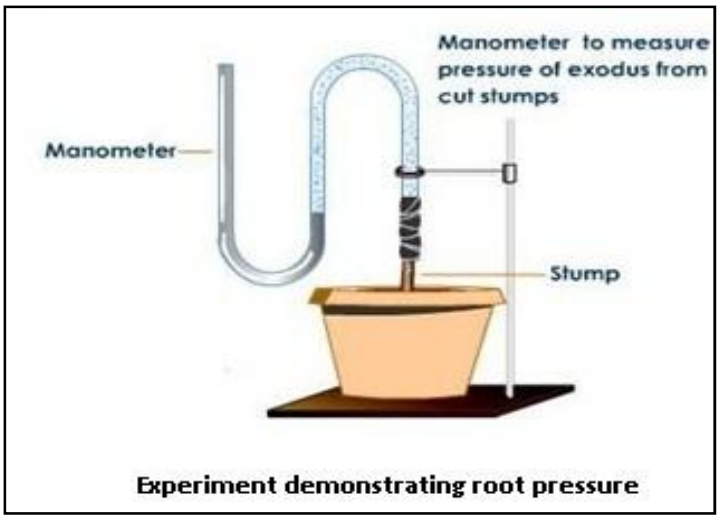
nonliving continuum, which included all the nonliving cells and cell walls of xylem as the apoplast. Plant scientists also consider apoplast a system that includes all nonliving cells and all walls and intercellular spaces in roots and shoots (stems and leaves) where water and dissolved solutes might translocate. Since living cells are not included, water translocation within this system is not directly due to osmosis per second but to the capillary action or as with solutes, to free diffusion.

Movement of water and solutes into the living cells of the plant is due to osmosis (water), free diffusion (passive uptake of solutes) or active uptake (solutes). This living continuum in the plant, including the plasmodesmata and elements within the cytoplasmic membrane, Munch termed the symplast.

Water that is absorbed moves from the soil to the interior of the root along an increasingly negative water potential gradient which is movement of water through the root epidermis and into the cortex because of increasing energy gradients established by proportional solute concentration .A theory of Crafts and Broyer (1938) suggests that there is a decreasing $O_2$ and increasing $CO_2$ gradient from the cortex to the stele. Metabolic activity would be a minimum, then, in the interior cells in the immediate area of the xylem ducts.

## Root pressure

In the root pressure we may observe xylem sap under pressure exuding from the cut end of the stump. If a well watered tomato plant is detopped and the stump is attached with a rubber sleeve to a manometer, a rise in the level of liquid in manometer can be demonstrated as a result of root pressure. Stocking (1956) defined the root pressure as a pressure developing in the



Experiment demonstrating root pressure

tracheary elements of xylem resulting from the metabolic activities of roots. Root pressure is referred to as an active process. The movement of water up to the stem as result of root pressure is due to osmotic mechanisms that are created as a result of the active absorption of salts by the roots.

Root pressure which is developed due to the accumulation of solute in the xylem ducts appears to be affected by factors that affect respiration Viz; oxygen tension, narcotics, auxin and respiration inhibitors. Several investigations have observed an automatic, diurnal fluctuation in exudations caused by root pressure. Dropped tomato plants with their root systems immersed in solutions of different concentrations exhibit different exudation rates. Lower exudation rates result when roots are immersed in solutions of lower concentration. Vaadia (1960) has suggested that the diurnal fluctuation of exudation rates is caused by the periodicity of salt transfer into the xylem. Obviously, this would cause a periodicity in the magnitude of the osmotic potential of the xylem ducts, which would have the effect of a change in the rate of water absorption in accordance with a change in water potential gradients. The water absorbed in this manner does not require a direct expenditure of energy. The energy is expended in the absorption and accumulation of salts. However, the water potential is the driving force in water absorption.

## Factors affecting water absorption

**Soil temperature:** The rate of absorption increases with rise in soil temperature up to 35 $^0$ C, thereafter it declined due to its effect on permeability of plasma membrane. Low temperature reduces the absorption of water. Therefore, cold soils are considered physiologically dry. Low temperature reduces the absorption of water due to following reasons.

- Increased viscosity of water which retards the water movement to the plant.
- Decrease in rate of root elongation and roots fail to reach new areas.
- Alternation in properties of protoplasm. Due to low temperature viscosity of protoplasm increases and permeability of plasma membrane to water decreases.

As a consequence the movement of water across the living cells of roots is slowed down.

- Reduction in metabolic activities of living cells**.**

**Soil air**: Low availability of $O_2$ in soil affects water absorption adversely. Soil lacking $O_2$ favours the action of anaerobic bacteria like Clostridium which decomposes soil matter and release poisonous gases like $H_2S$, ethylene, ammonia etc. which are toxic to roots and inhibit their development.

**Soil water:** Increase in water content of soil increases water absorption to a certain limit, thereafter it declines due to decrease in aeration of soil.

**Mineral salts:** Absorption of water is also retarded by higher concentration of salts in soil because they increase the osmotic pressure of soil solutions. Saline soils and salt marshes are physiologically dry for the plants.

## 6. LECTURE NOTES

# Mechanism of water uptake and transport by apoplastic and symplastic methods, factors affecting water absorption.

### Mechanism

Previously it was thought that absorption of mineral salts takes place along with water absorption. But it is now understood that mineral salt absorption and water absorption are two different processes.

Mineral salts are absorbed from the soil solution in the form of ions. They are chiefly absorbed through the meristematic regions of the roots near the tips.

Plasma membrane of the root cells is not permeable to all the ions. It is selectivity permeable. All the ions of the same salt are not absorbed at equal rate but leads unequal absorption of ions. First step in the absorption of mineral salts is the process of Ion exchange which does not require metabolic energy.

The further processes of the absorption of mineral salts may be of two types.

1. **Passive and 2. Active**

### 1. Passive absorption

When the concentration of mineral salts is higher in the outer solution than in the cell sap of the root cells, the mineral salts are absorbed according to the concentration gradient by simple process of diffusion. This is called as passive absorption because it does not require expenditure of metabolic energy.

### Ion exchange

The ions adsorbed on the surface of the plasma membrane of the root cells may be exchanged with the ions of same sign from external solution for eg. The cation $K^+$ of the external soil solution may exchanged with $H^+$ ions adsorbed on the surface of the plasma membrane. Similarly anion may be exchanged with OH ions. There are two theories regarding the mechanism of ion exchange.

### 1. Contact exchange theory

According to this theory the ions adsorbed or the surface of root cells and clay particles are not held tightly but oscillate within small volume of space. If the roots and clay particles are in close contact with each other, the oscillation volume of ions adsorbed on root surface may over by the oscillation volume of ions adsorbed on clay particles, and the ions adsorbed on clay

particle may be exchanged with the ions adsorbed on root surface directly without first being dissolved in soil solution.

**2. Carbonic acid exchange theory**

According to this theory, the $CO_2$ released during respiration of root cells combines with water to form carbonic acid ($H_2CO_3$). Carbonic acid dissociates into $H^+$ and an anion $HCO_3$ in soil solution. These $H^+$ ions may be exchanged for cations adsorbed on the clay particles. The cations thus released into the soil solution from the clay particles, may be adsorbed on root cells in exchange for $H^+$ ions or as in ion pairs with bicarbonate. Thus, the soil solution plays an important role in carbonic acid exchange theory.

**2.1 Active absorption of mineral salts**

It has been observed that the cell sap in plants accumulates large quantities of mineral slats ions against the concentration gradient. The accumulation of mineral salts against to concentration gradient is an active process which involves the expenditure of metabolic energy through respiration. The active absorption of mineral salts involves the operation of a carrier compound present in the plasma membrane of the cells.

The carrier concept

According to this theory, the plasma membrane is impermeable to free ions. But some compounds present in it acts as carrier and combines with ions to form carrier- ion- complex which can move across the membrane. On the inner side of the membrane this complex leaves releasing ions into the cell while the carrier goes back to the outer surface to pick up fresh ions.

They are two hypotheses based on the carrier concept to explain the mechanism of active salt absorption. Although they are not universally accepted.

**1. Lundegardhs cytochrome pump theory**

Lundegardh and Burstrom (1933) believed that there was a definite correlation between respiration and anion absorption. Thus when a plant is transferred from water to a salt solution the rate of respiration increases. This increase in rate of respiration over the normal respiration has been called as anion respiration or salt respiration.

Lundegardh (1954) proposed cytochrome pump theory which is based on the following assumptions.

1. The mechanism of anion and cation absorption is different
2. Anions are absorbed through cytochrome chain by an active process.

(Cytochromes are ion – porphyrin proteins that act as enzymes and helps in election transfer during respiration).

3. Cations are absorbed passively.

According to this theory

1) Dehydrogenase reactions on inner side of the membrane give rise to protons ($H^+$) and electrons ($e^-$).
2) The electrons travels over the cytochrome chain towards outside the membrane, so that the Fe of the cytochrome becomes reduced ($Fe^{++}$) on the outer surface and oxidized ($Fe^{+++}$) on the inner surface.
3) On the outer surface, the reduced cytochrome is oxidized by oxygen releasing the electron ($e^-$) and taking an anion ($A^-$).
4) The electron thus released unites with $H^+$ and oxygen to form water
5) The anion ($A^-$) travels over the cytochrome chain towards inside.
6) On the inner surface the oxidized cytochrome becomes reduced by taking an electron produced through the dehydrogenase reactions and the anion (A) is released.
7) As the result of anion absorption, a cation (M) moves passively from outside to inside to balance the anion.

**2. Bennert – Clark's protein Lecithin Theory**

In 1856, Bennet – Clark suggested that because the cell membranes chiefly consist of phospholipids and proteins and certain enzymes seem to be located on them, the carrier could be a protein associated with the phosphatide called as lecithin. He also assumed the presence of different phosphatides to correspond with the number of known competitive groups of cations and anions.

**According to this theory**

1. Phosphate group in the phosphatide is regarded as the active centre binding the cations and the basic choline group as the anion binding centre.
2. The ions are liberated on the inner surface of the membrane by decomposition of lecithin by the enzyme lecithinase.
3. The regeneration of the carrier lecithin form phosphatidic acid and choline takes place in the presence of the enzyme choline acetylase and choline esterase and ATP. The latter acts as a source of energy.

**Donnans' Equilibrium**

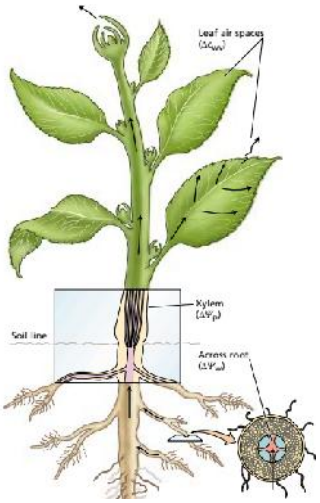
The accumulation of ions inside the cells without involving expenditure of the metabolic energy can be explained to some extent by Donnan's equilibrium theory.

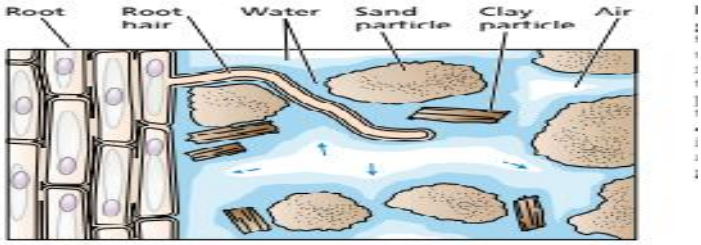
According to this theory there are certain pre existing ions inside the cell which cannot diffuse outside through membrane. Such ions are called as in diffusible or fixed ions. However, the membrane is permeable to both anions and cations of the outer solutions.

Suppose there are certain fixed anions in the cell which is in contact with outer solution containing anions and cations. Normally equal number of anions and cations would have

diffused into the cell through an electrical potential to balance each other, but to balance the fixed anions more cations will diffuse into the cell. This equilibrium is known as Donnan's equilibrium. In this particular case, there would be an accumulation of cations inside the cell.

If however, there are fixed cations inside the cell, the Donnan's equilibrium will result in the accumulation of anions inside the cell.




**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

In sandy soils, the spaces between particles are so large that water tends to drain from them and remain only on the particle surfaces and at interstices between particles. In clay soils, the channels are small enough that water does not freely drain from them; it is held more tightly . The moisture-holding capacity of soils is called the field capacity. Field capacity is the water content of a soil after it has been saturated with water and excess water has been allowed to drain away. Clay soils or soils with a high humus content have a large field capacity. A few days after being saturated, they might retain 40% water by volume. In contrast, sandy soils typically retain 3% water by volume after saturation. In the following sections we will examine how the negative pressure in soil water alters soil water potential, how water moves in the soil, and how roots absorb the water needed by the plant. A Negative Hydrostatic Pressure in Soil Water Lowers Soil Water Potential Like the water potential of plant cells, the water potential of soils may be dissected into two components, the osmotic potential and the hydrostatic pressure. The osmotic potential of soil water is generally negligible because solute concentrations are low; a typical value might be –0.02 MPa. For soils that contain a substantial concentration of salts, however, Ys is significant, perhaps –0.2 MPa or lower. The second component of soil water potential is hydrostatic pressure (Yp).

**WATER ABSORPTION BY ROOTS**

Intimate contact between the surface of the root and the soil is essential for effective water absorption by the root. This contact provides the surface area needed for water uptake and is

maximized by the growth of the root and of root hairs into the soil. Root hairs are microscopic extensions of root epidermal cells that greatly increase the surface area of the root, thus providing greater capacity for absorption of ions and water from the soil. When 4-month-old rye (Secale) plants were examined, their root hairs were found to constitute more than 60% of the surface area of the roots. Water enters the root most readily in the apical part of the root that includes the root hair zone. More mature regions of the root often have an outer layer of protective tissue, called an exodermis or hypodermis, that contains hydrophobic materials in its walls and is relatively impermeable to water. The intimate contact between the soil and the root surface is easily ruptured when the soil is disturbed. It is for this reason that newly transplanted seedlings and plants,need to be protected from water loss for the first few days after transplantation. Thereafter, new root growth into the soil reestablishes soil–root contact, and the plant can better withstand water stress. Let's consider how water moves within the root, and the factors that determine the rate of water uptake into the root. Water Moves in the Root via the Apoplast, Transmembrane, and Symplast Pathways In the soil, water is transported predominantly by bulk flow. However, when water comes in contact with the root surface, the nature of water transport becomes more complex.

**From the epidermis to the endodermis of the root, there are three pathways through which water can flow: the apoplast, transmembrane, and symplast pathways**.

1. In the apoplast pathway, water moves exclusively through the cell wall without crossing any membranes. The apoplast is the continuous system of cell walls and intercellular air spaces in plant tissues.

2. The transmembrane pathway is the route followed by water that sequentially enters a cell on one side, exits the cell on the other side, enters the next in the series, and so on. In this pathway, water crosses at least two membranes for each cell in its path (the plasma membrane on entering and on exiting). Transport across the tonoplast may also be involved.

3. In the symplast pathway, water travels from one cell to the next via the plasmodesmata . The symplast consists of the entire network of cell cytoplasm interconnected by plasmodesmata. Although the relative importance of the apoplast, transmembrane, and symplast pathways has not yet been clearly established, experiments with the pressure probe technique indicate that the apoplast pathway is particularly important for water uptake by young corn roots (Frensch et al. 1996; Steudle and Frensch 1996).

At the endodermis, water movement through the apoplast pathway is obstructed by the Casparian strip. The Casparian strip is a band of radial cell Apoplast pathway Symplastic and transmembrane pathways Epidermis Cortex Endodermis Casparian strip Pericycle Xylem Phloem Pathways for water uptake by the root. Through the cortex, water may travel via the apoplast pathway, the transmembrane pathway, and the symplast pathway. In the symplast pathway, water flows between cells through the plasmodesmata without crossing the plasma

membrane. In the transmembrane pathway, water moves across the plasma membranes, with a short visit to the cell wall space. At the endodermis, the apoplast pathway is blocked by the Casparian strip. walls in the endodermis that is impregnated with the waxlike, hydrophobic substance suberin. Suberin acts as a barrier to water and solute movement. The endodermis becomes suberized in the nongrowing part of the root, several millimeters behind the root tip, at about the same time that the first protoxylem elements mature (Esau 1953). The Casparian strip breaks the continuity of the apoplast pathway, and forces water and solutes to cross the endodermis by passing through the plasma membrane. Thus, despite the importance of the apoplast pathway in the root cortex and the stele, water movement across the endodermis occurs through the symplast.

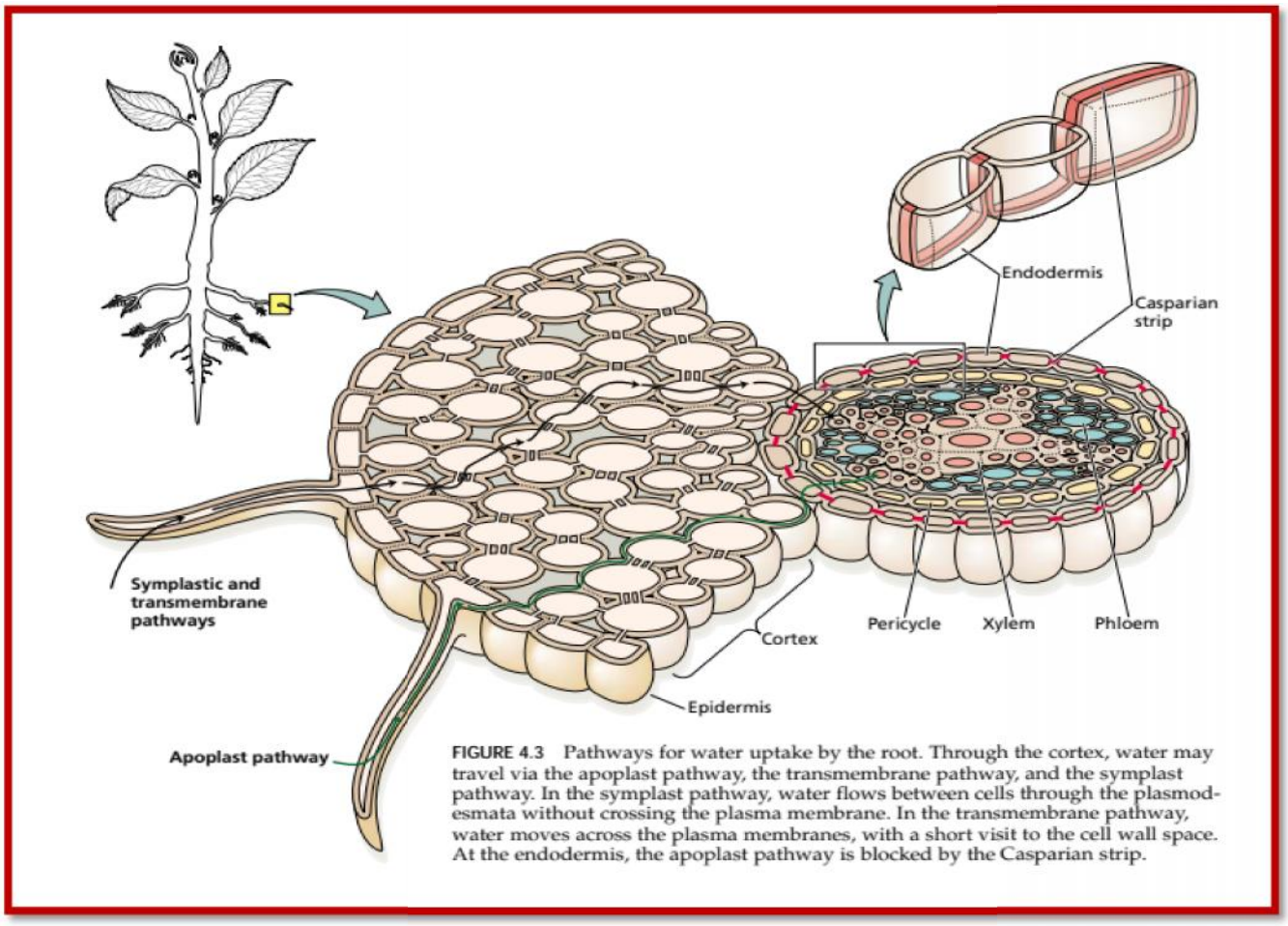



FIGURE 4.3 Pathways for water uptake by the root. Through the cortex, water may travel via the apoplast pathway, the transmembrane pathway, and the symplast pathway. In the symplast pathway, water flows between cells through the plasmodesmata without crossing the plasma membrane. In the transmembrane pathway, water moves across the plasma membranes, with a short visit to the cell wall space. At the endodermis, the apoplast pathway is blocked by the Casparian strip.

**Source :** Plant physiology. 3rd edn. L. Taiz and E. Zeiger.

## 7. LECTURE NOTES

**Transpiration Mechanism of transpiration, driving force, soil-plant-atmosphere continuum, advantages of transpiration, factors affecting transpiration, anti-transpirants.**

# TRANSPIRATION

## Transpiration

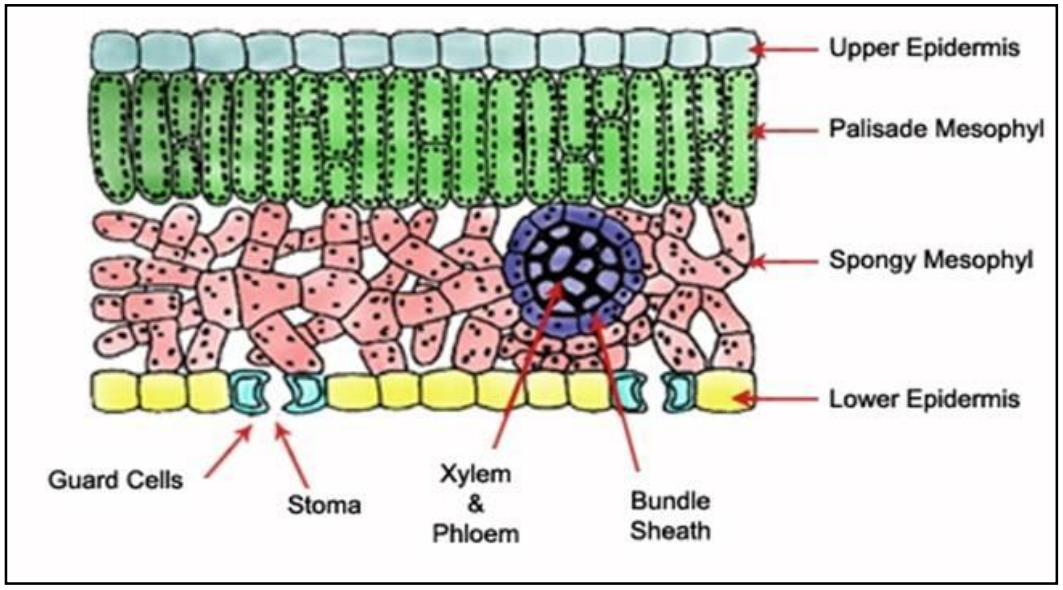
Diffusion of water vapours from aerial parts of the plants to the ambient atmosphere occurs through stomata, cuticle and lenticels is called transpiration. It is a vital phenomenon and regulated by cells, whereas, evaporation is a physical phenomenon. Generally 20-50 pounds of water is transpired for every ounce of dry matter produced.

## Kinds of transpiration

Most of the transpiration takes place through leaves also called foliar transpiration. Foliar transpiration is of two kinds stomatal and cuticular. Some transpiration also takes place through lenticels called lenticular transpiration.

## Mechanism

The transpiration takes place through stomata when the walls of mesophyll cells become saturated with water.



Between upper and lower epidermis of leaf mesophyll cells exist which consists of palisade cells and spongy parenchyma cells. The spongy parenchyma cells are connected with the atmosphere by means of stomata mostly found in lower epidermis. The xylem of leaf veins supply water to the mesophyll cells by osmotic diffusion. They become turgid and saturated with water. Water evaporates from then moist cells into internal atmosphere of intercellular spaces of mesophyll which becomes saturated with water. The outer atmosphere is usually unsaturated. This results in the diffusion of water from intercellular spaces of leaf into outer atmosphere through stomata. This is referred as stomatal transpiration .Due to loss of water the concentration of water vapour into intercellular spaces decreases which results in the more evaporation of water from mesophyll cells. Some transpiration also takes place by direct evaporation from the outer walls of epidermal cells through cuticle. This constitutes cuticular transpiration. The cuticle being impervious to water the amount of water loss is comparatively less between 3-10%. In stems, fruits and flower parts the transpiration is mostly cuticular. In herbaceous plans where cuticle is poorly developed cuticular transpiration almost equals stomatal transpiration. In woody stems some transpiration also takes place through lenticels (certain cracks developed on the bark) .This constitutes lenticular transpiration.

## Driving force in transpiration

- Difference in water vapour concentration between leaf mesophyll cells (air space) and external air.
- Diffusional resistance of this pathway which includes resistance associated with diffusion through stomatal pore and leaf boundary layer resistance depends on layer of unstirred air next to the leaf surface through which water vapour must diffuse to reach turbulent air of atmosphere.

## Soil – plant – atmosphere continuum

It includes the following:

- In the soil and xylem water moves by bulk flow in response to a pressure gradient ($\Delta \Psi p$).
- In a vapour phase water moves primarily by diffusion, at least until it reaches the out side leaf, where convection (a form of bulk flow) becomes dominant.
- When water is transported across membranes, the driving force is water potential difference across the membrane. Such osmotic flow occurs when cells absorb water and when roots transport water from soil to the xylem.
- In all of these situations water moves towards region of low water potential or free energy.

## To increase $CO_2$ uptake and reduce transpiration plants must have:

- An extensive root system to extract the water from the soil.
- A low resistance pathway through xylem vessel and tracheids to bring water to the leaves.
- A hydrophobic cuticle covering surfaces of the plant to reduce evaporation.
- Microscopic stomata on the leaf surface to allow gas exchange.
- Guard cells to regulate the diameter (and diffusional resistance) of stomata aperture.

## Advantages of transpiration

- It creates a suction force to absorb water and minerals from the soil.
- The main force in ascent of sap is brought about by transpirational pull.
- It affects DPD thus helps diffusion through cells.
- Plants generally absorb excess quantities of water. If this water is not transpired it will disturb the osmotic balance between cells and also bring about decay of tissues. Transpiration stream helps in solute transport from one part of plant to another.
- Transpiration stream helps in the translocation of solutes from one part of the plant to another.

- Metabolic activities in plants increases the temperature and transpiration brings down the temperature. Thus the temperature within the plant is maintained.
- Opening and closing of stomata during transpiration indirectly influences the photosynthesis and respiration.
- It enhances the movement of molecules within the plants.

## Disadvantages

In some soils where water availability is in scarcity the excess transpiration may even kill the plant. It has been found out that plant cells can maintain their turgidity even in absence of transpiration.

## Anti-transpirants

Substances which reduce transpiration rate by causing stomatal closure partially. Examples - Colourless plastics, silicone oil, low viscosity waxes, abscisic acid, $CO_2$ when concentration increases from 0.03% to 0.05%.

## Magnitude of transpiration

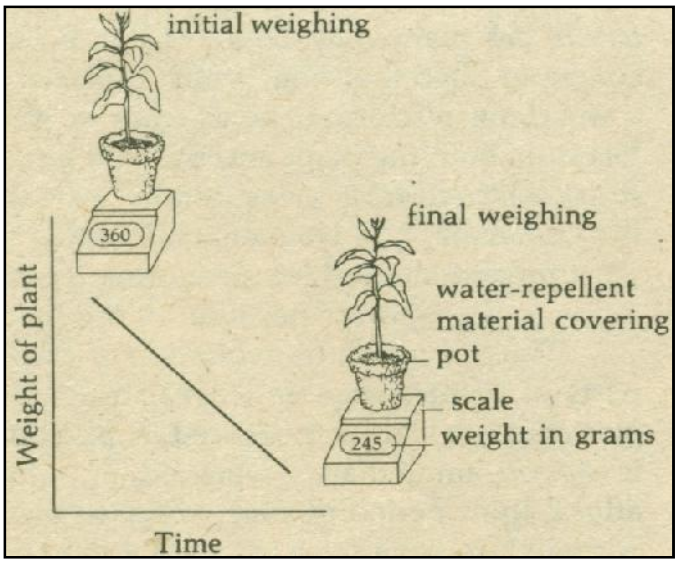
The amount of water a plant actually uses is small as compared to the large quantities it transpires. Transpiration rates of some herbaceous plants are so great that, under favourable conditions, the entire column of water in a plant may be replaced in the course of the single day (Stiles 1924).For example, it has been estimated that a single corn plant may transpire up to 54 gallons of water in a growing season. At this rate, a single acre of corn could transpire the equivalent of 15 inches of water during one growing season. The amount of water loss varies from species to species.

## Measurement of transpiration

Several methods are being used to measure the transpiration rates. These methods either measure the water absorbed or the water vapour transpired by the plant. The first approach takes advantage of the accordance between the absorption and transpiration rates under most conditions.

## Weighing method

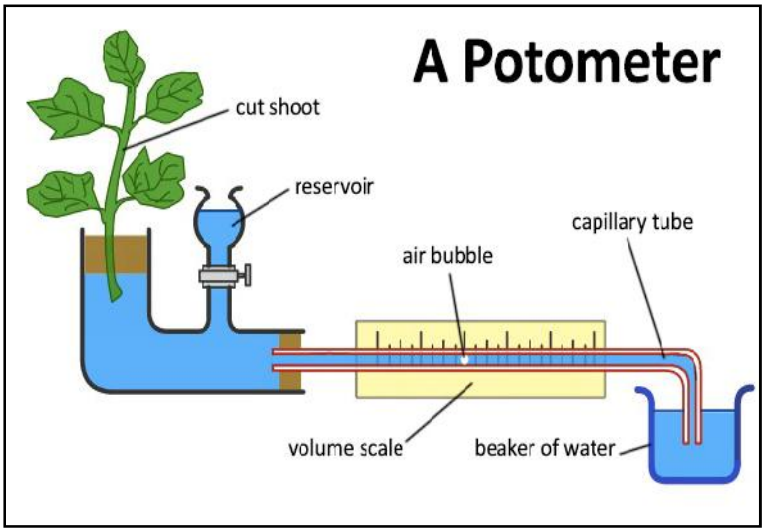
Simplest way to measure the transpiration is to weigh a potted plant at the beginning and at the end of the prescribed period of time. The soil surface should be covered and the pot wrapped with some water repellent material such as aluminum foil to retard evaporation from surfaces other than the plant. The loss of the weight by the plant over a period of time will be almost completely due to transpiration. Gain and loss of weight by the plant over a short period of time will be almost completely due to transpiration. Gain or loss of weight due to photosynthesis or respiration is insignificant. When using this method, we are restricted to small plants that can be conveniently grown in a pot. For field work, plant scientists often use a very large balance known as lysimeter. A big plant may be grown in a large container filled with soil, which is placed on a weighing platform. The amount of water loss from both the plant and the soil is termed as evapotranspiration and is measured by weighing the container. The lysimeter is the most precise method for measuring transpiration and evapotranspiration in field.




The amount of transpiration of excised parts of plants such as leaves, fruits and branches can also be measured. A plant part is excised, immediately weighed and then after a short period of time weighed again. Although relative rates of transpiration may be compared in this manner, transpiration of excised organ frequently deviates from the normal transpiration of the intact plant. In the initial stages, the rate of transpiration of an excised organ may exceed normal rates, probably because of the release of the tension in the xylem ducts. After a short period of time, however, transpiration rates will fall off

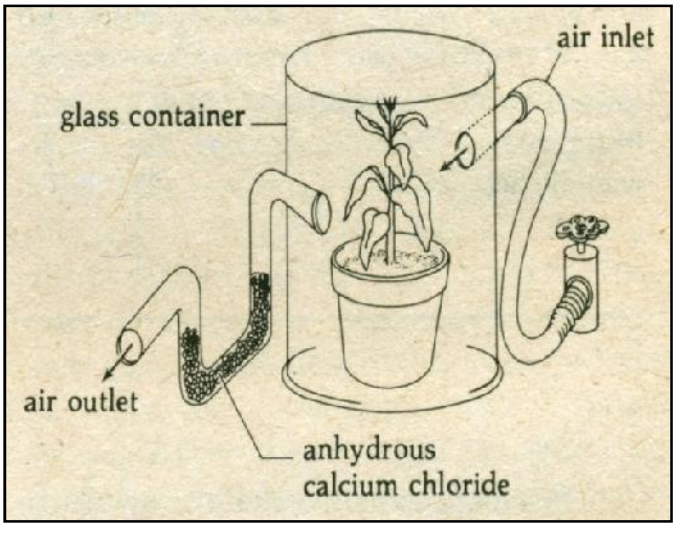
because of decrease in the water content of the tissue, stomatal closure, permeability changes etc.

## Potometer method




This method is based on the premise that the rate of water absorption is very nearly equal to the rate of transpiration. A portion of a shoot of a suitable plant is sealed in a water filled glass vessel which has two other outlets, a graduated capillary tube and a water reservoir. Before the measurement of transpiration, the entire apparatus is filled with water so that no air spaces are present. This can be managed by manipulating the stopcock, which controls the flow of water into the vessel from the reservoir. An air bubble is then introduced into the capillary tube. As transpiration proceeds, the air bubble will move along the capillary tube and give a measure of the rate of transpiration. The potometer method is ideal for observing the effects of different environmental factors like temperature, light, air movement etc. on transpiration rates. However, its utility is limited because it actually measures water absorption rather than transpiration, under certain circumstances the two can vary considerably.

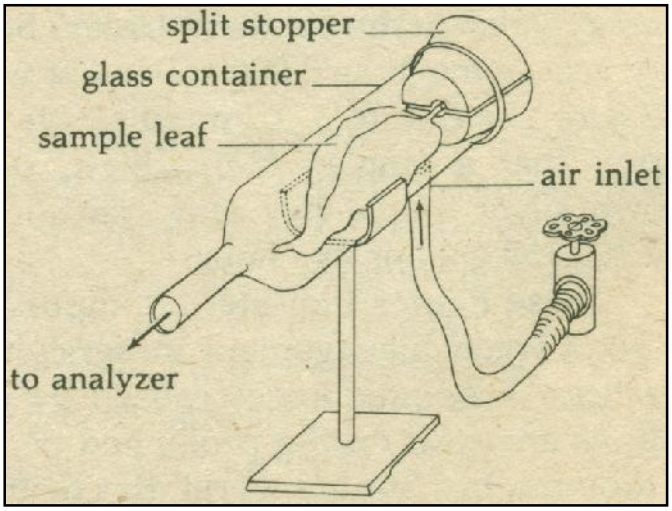
## Collection and weighing of water vapour method




Transpiration may be measured by enclosing the plant in a glass container, so that the water vapours can be trapped and weighed. Air of known moisture content is passed over the

plant through an opening in the glass container and passed out over some preweighed water absorbing material, such as anhydrous calcium chloride. The continuous stream of air passing over the plant keeps the moisture content of the enclosed air approximately equal to that of the surrounding atmosphere. The moisture content of the air passed over the plant is measured by passing it through the same apparatus minus the plant. The difference in weight between the calcium chloride before and after air is passed through , is a measure of the moisture content of the air. The difference in weight between the calcium chloride receiving the air passed over the plant and the calcium chloride receiving the air passed through the apparatus without the plant is a measure of transpiration.

## Cuvette method

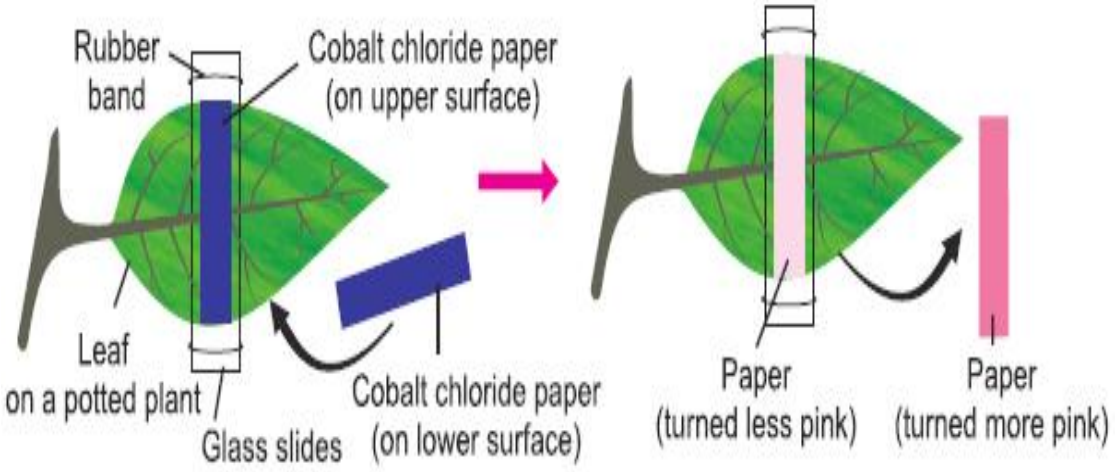



This method is designed to measure transpiration of a single leaf. This method is ideal for the laboratory work when the experimenter is interested in following the effect of different factors like light, temperature and humidity on the transpiration process. Air of the known humidity is introduced into the cuvette, passed over the leaf and collected after it exits. The relative humidity is determined and provides a measure of the rate of transpiration. This method is useful only in laboratory, but not in the field , scientists often use tent chambers equipped with suitable built in air inlets and outlets and temperature sensing devices to measure transpiration of large plants. Air of known water content is passed into the tent and over the plant. As the water exits, its relative humidity is measured. The increase in water content in the air is a good measure of the transpiration process.

## Cobalt chloride

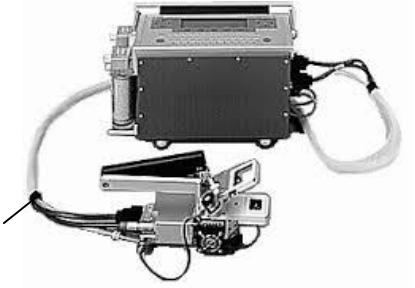
In this method, transpiration is indicated by a range of colour rather than change in weight. Filter paper disks are impregnated with a slightly acidic 3 percent solution of cobalt chloride and thoroughly dried. When dry paper impregnated in this manner will be blue in colour, when the paper is exposed to humid air, it will gradually change in pink. In a similar manner, the cobalt chloride treated paper will gradually change from blue to pink when exposed to a transpiring leaf surface. The rate of colour change is indicative of the rate of transpiration.

The cobalt chloride method can be used for measuring only the relative rates of transpiration of different plants. Due to modifications of different environmental conditions, transpiration rates indicated by this method may deviate considerably from the actual transpiration rates. The surface of the leaf covered by the paper is subjected to practically no air movement, a reduction in light and a steeper vapour pressure gradient.




## Infra red gas analyser

Transpiration rate can also be recorded by using Infrared gas analyzer.



**Infrared gas analyser**

## Factors affecting transpiration

## External

## Humidity of air

Transpiration takes place through the diffusion of water vapours from the intercellular spaces of leaf mesophyll cells into outer atmosphere through stomata. Diffusion only will take place if the water vapour content of atmosphere is less than intercellular spaces of leaf. Atmosphere saturated with water vapours favour less loss of water from the leaves. In dry weather transpiration rate becomes higher.

The transpiration rate depends upon capacity of atmosphere to take up more moisture which also depends upon the difference between the amount of water actually present in air and amount necessary to completely saturate it. This difference is called saturation deficit. The amount of moisture actually present in air is called absolute humidity, whereas, amount of moisture necessary for saturation at a particular temperature is called relative humidity.

## Temperature

An increase in temperature causes an increase in rate of the transpiration. Low temperature decreases the capacity of air to hold moisture and consequently increases the relative humidity and decreasing the rate of transpiration. High temperature causing the stomata to open widely which accelerates the transpiration.

## Wind

The wind removes air moisture from vicinity of plant and mixing it with dry air. This brings down the humidity of air in contact with transpiring surface and transpiration is promoted.

## Light

Light affects transpiration by two ways 1. By raising the temperature of leaves 2. By causing the stomatal opening.

## Atmospheric pressure

Low air pressure increases the rate of transpiration through reduction in air density. But in nature this condition happens at higher altitudes having low temperatures which neutralizes the effect of reduced pressure on transpiration.

## Available soil water

The transpiration rate can be maintained only if enough water is absorbed by roots from the soil to compensate the loss. The factors which affect absorption of water indirectly affect the rate of transpiration.

## Internal factors

Water relation of parenchyma cells influences the transpiration rate. The mesophyll cells lose water under saturated conditions. If this loss is not compensated through absorption of water from the root a water deficit in leaf cells is created. Water content of mesophyll cells then decreases resulting in loss of turgidity. The cell wall becomes dry and transpiration is reduced. Under these conditions osmotic pressure of mesophyll cells increases resulting in withdrawal of water from guard cells to mesophyll cells. The stomata closes due to loss of turgidity of guard cells. Among other factors are number of stomata per unit area of leaf surface and their position. In some plants like oleander and pinus the stomata are sunken in depressions below the epidermis so that they are safe from effect of wind action.

## Daily and seasonal periodicity of transpiration

Transpiration rate begins to rise very rapidly from early morning onwards then increases gradually reaching the maximum at about mid day, thereafter declines slowly and minimum is obtained at about sunset and it further declines during night hours due to closure of stomata. The periodicity is the result of interaction of two factors, the diurnal movement of stomata and external conditions influencing the transpiration.

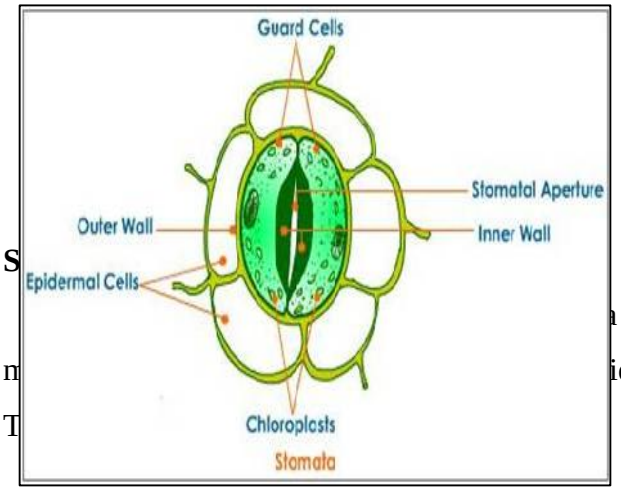
## 8. LECTURE NOTES

**Stomatal physiology: Structure of stomata and mechanism of opening and closing, classification of stomata, theories of mechanism of opening and closing of stomata, bleeding, guttation.**

## Structure of stomata and mechanism of opening and closing

Stomata are important structures which facilitates transpiration and gaseous exchange during photosynthesis and respiration. They are minute structures generally elliptical (oval in shape) and found mostly in lower surface of leaf. A stoma is covered with two epidermal cells called guard cells. They are smaller in size than other epidermal cells. In dicots they are kidney shaped. In some species examples grasses adjacent to guard cells some specialized structures exist called accessory or subsidiary cells. A stoma contains nucleus, vacuoles and chloroplasts.

Stomata open with increase in turgidity of guard cells and with decrease in turgidity stomata become narrow and closes. The walls surrounding the pore are thick and inelastic and resists stretching. The outer walls are thin, elastic and are stretched when subjected to pressure. When water enters guard cells they become turgid . The outer thin walls are stretched, while inner thick walls fail to stretch and are pulled away and become concave. This increases the gap between the guard cells resulting in opening of stomata. With the loss of turgor, the inner walls of guard cells become straight and approach each other, the pore becomes narrow and finally closes. Light, external $CO_2$ concentration and water content of leaf cells affect stomatal opening. Generally stomata open in light and close in dark.

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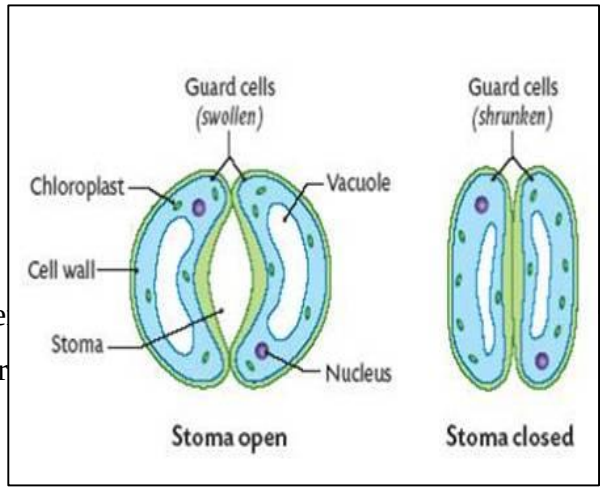


$$I = \frac{S}{E+S} \, x \, 100$$

Where I = Stomatal index, E = Number of epidermal cells, S = Number of stomata / unit area.

The size of stomatal aperture varies from 7x3 µ in *Phaseolus vulgaris* to 38 x 8 µ in *Avena sativa*. The number of stomata / sqm. of leaf surface varies between 50- 300 .

**Classification of stomata**

According to position and distribution the stomata can be classified as under:

**Apple and mulberry type –** Found only on upper leaf surface. Examples – peach, walnut, nasturtium, oxalis, yew etc.

**Potato type -** Mostly on under surface. Examples - cabbage, bean, pea and tomato.

**Oat type –** Almost equal distribution on both surfaces. Examples – wheat, maize and most of the cereals.

**Water lily type -** Only on upper surface. Examples - aquatic plants with floating leaves.

**Potamogeton type –** Stomata almost absent if present then remains functional less. Examples – submerged plants.

**Theories of mechanism of opening and closing of stomata**

**Starch – sugar hypothesis**

Given by J.D. Sayre (1926); Scarth (1932) and Small and Clarke (1942). In dark $CO_2$ which is not utilized in photosynthesis found dissolved in guard cells forming the carbonic acid ($H_2CO_3$) due to this $_pH$ decreases. In day photosynthesis starts due to light in guard cells carbonic acid is removed, as a result $_pH$ of guard cells increases. Starch formed during night converted to glucose 1 phosphate. Starch is insoluble and osmotically inactive but glucose phosphate is soluble and increases osmotic pressure of guard cells. As a result of this water will enter from surrounding cells to guard cells increasing the turgor pressure of guard cells resulting in opening of stomata.

pH 7

Starch +ip ⟶ Glucose 1 Phosphate

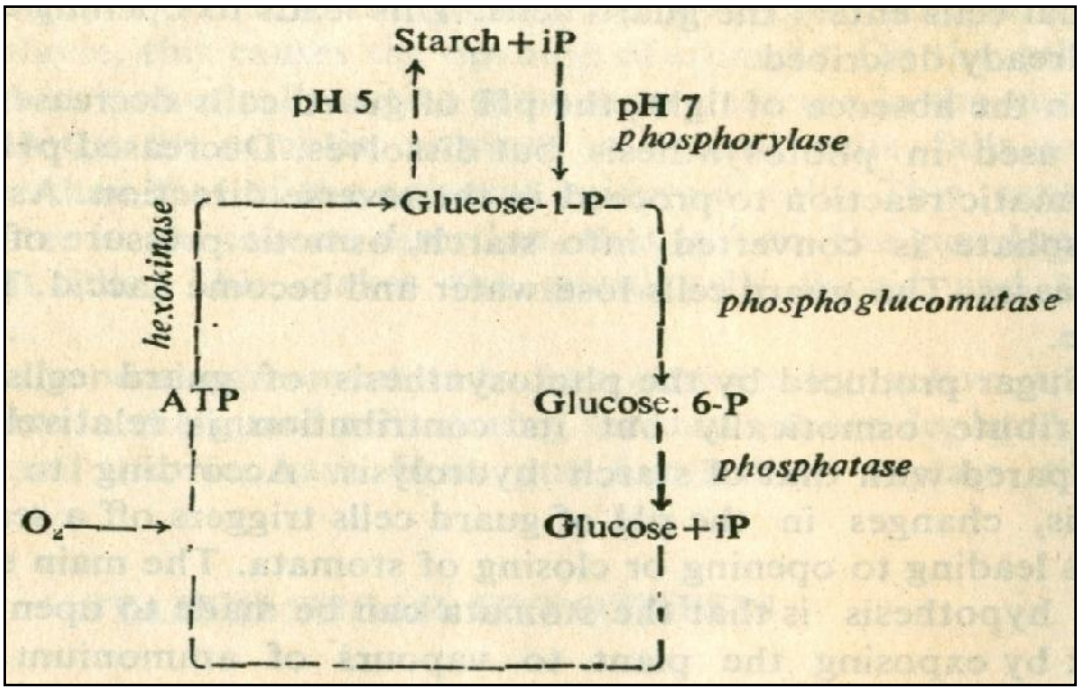
Starch phosphorylase ⟵

pH 5

**Steward's (1964) scheme**

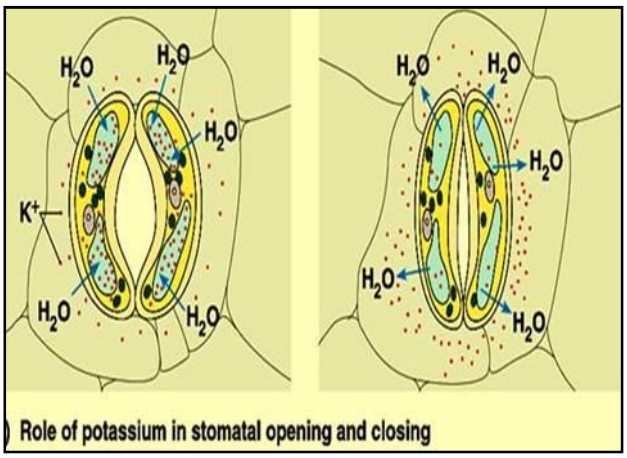
Steward had proposed some modified scheme over Starch – sugar mechanism.

Glucose 1 phosphate splits into glucose and ip both are osmoticlly active and together give to the guard cells a higher osmotic pressure. During closing of stomata in dark glucose converted back to glucose 1 phosphate. This transformation requires $O_2$ and metabolic energy in the form of ATP.

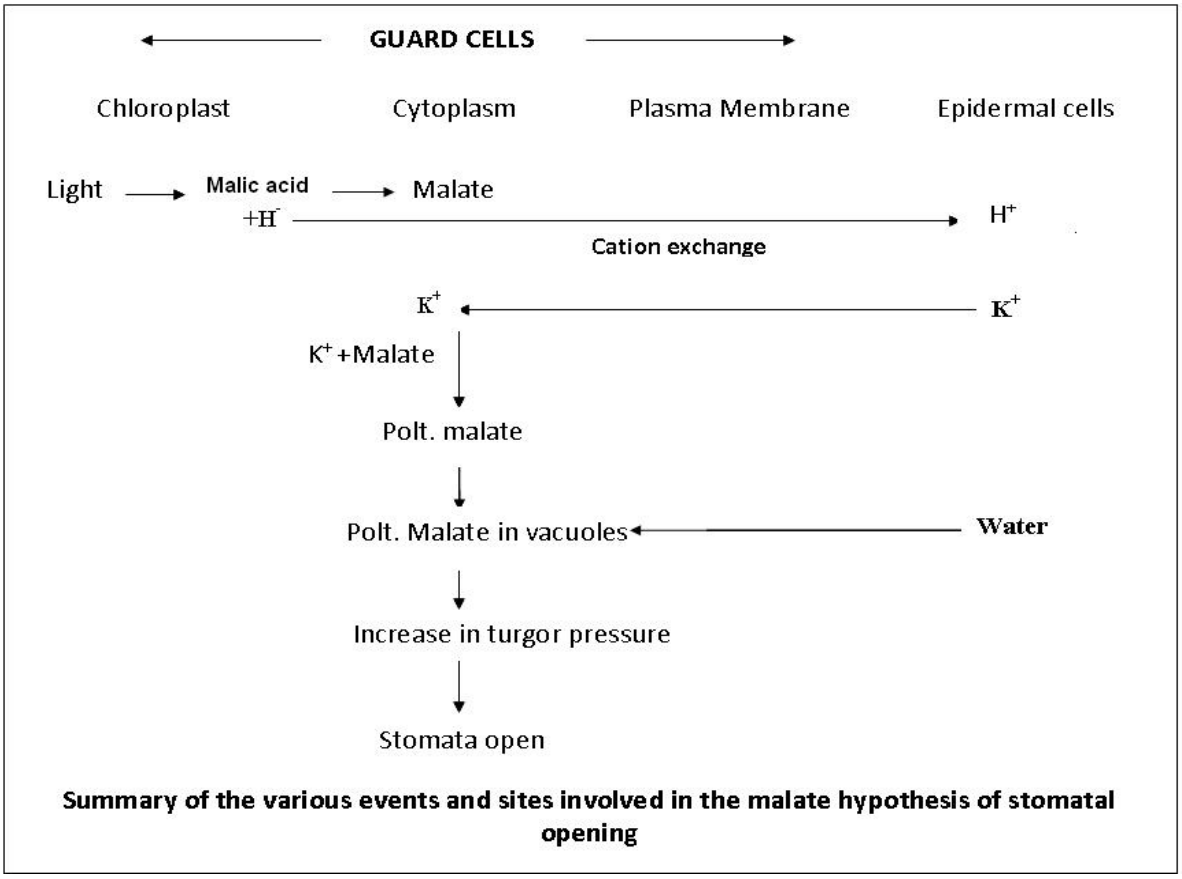



**Malate hypothesis or potassium ion exchange theory**

This is also called Potassium ion exchange theory. According to this theory (Levitt 1974) starch produces malic acid during respiratory pathway in presence of light in chloroplast. Malic acid is then excreted into cytoplasm of guard cells. It is a weak acid therefore dissociates into Malate and $H^+$ ions. The $H^+$ ions are removed from guard cells into surrounding cells and $K^+$ ions take place of them in guard cells. This increases the osmotic pressure of guard cells. In dark $H^+$ ions would enter into guard cells replacing the $K^+$ ions. $H^+$ ions combines with malate to form malic acid which is used in respiration.



Role of potassium in stomatal opening and closing



**Summary of the various events and sites involved in the malate hypothesis of stomatal opening**

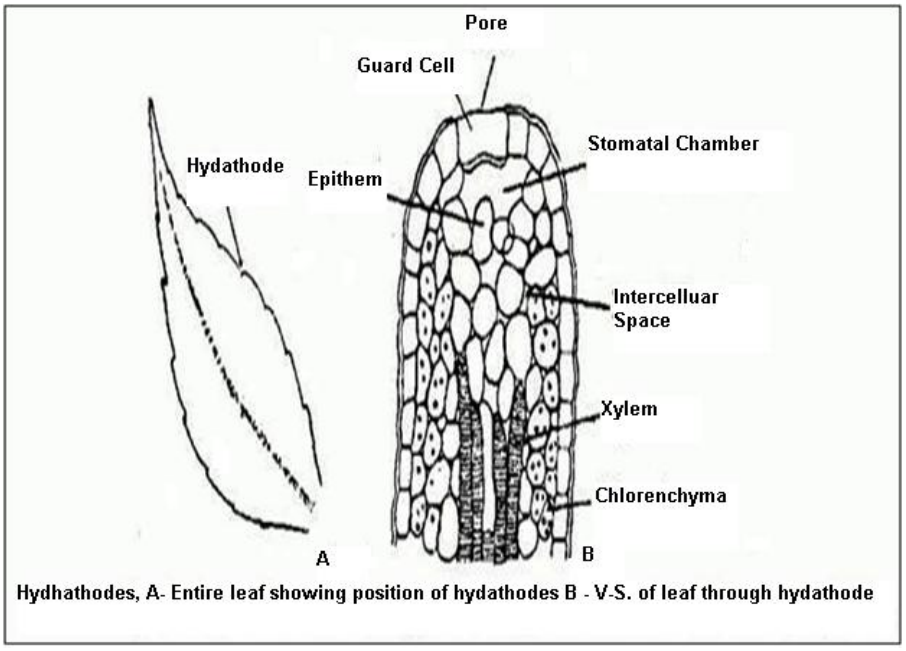
**Stomatal movement in succulent plants**

Succulent plants like Bryophyllum, Cactus form malic acid in night which increases the osmotic pressure of guard cells which results in opening of stomata. During the day the malic acid disappears. Due to formation of malic acid in dark $_{P}$Hshould become low and stomata should remain closed but malic acid being strong solute increases the osmotic pressure of guard cells resulting in opening of stomata. In such plants starch sugar mechanism may not operate.

**Bleeding**

Exudation of liquid water, sap and dissolved substances from injured part of plant.

**Guttation**



Hydhathodes, A- Entire leaf showing position of hydathodes B - V-S. of leaf through hydathode

Oozing of water drops from leaf tip where principal vein ends. Conditions that promote active absorption of water and hinder transpiration favour guttation. Phenomenon is seen in oat, potato, tomato, garden nasturtium and grasses. The water of guttation contains carbohydrates,nitrogenous compounds, organic acids and mineral salts. Guttation takes place from more are less spherical structures called hydathode or water stomata. They are commonly found in plants inhabiting humid tropics. It consists of large sized pore which always remains open. Beneath the pore is an air cavity. Below it a loose tissue called epithem made up of small cells without chlorophyll. Underneath this are tracheids. The liquid is forced out of tracheids into intercellular spaces of epithem by root pressure.

**9. LECTURE NOTES**

**Mineral nutrition of plants: Criteria of essentiality of elements, essential elements, Physiological role of elements in plants in plants. Method of detection of elements, deficiency symptoms of elements in plants**

# MINERAL NUTRITION

## Criteria of essentiality of elements

Many scientists have given the criteria on that basis the elements are designated as essential elements as described below:

## Arnon and Stout (1939)

- The element must be essential for growth and reproduction of plant. Its absence hinders such activities.
- The requirement for the element must be specific and can not be replaced by any other element.
- The element must be acting directly inside the plant not simply causing other element to be readily available or reversing the toxic effect of other elements.

## Epstein (1972)

- An element is essential, if plant can not complete its life cycle due to its absence.
- An element is essential, if it is a part of molecule which itself is essential in plants like nitrogen in protein and magnesium in chlorophyll.

## Essential elements

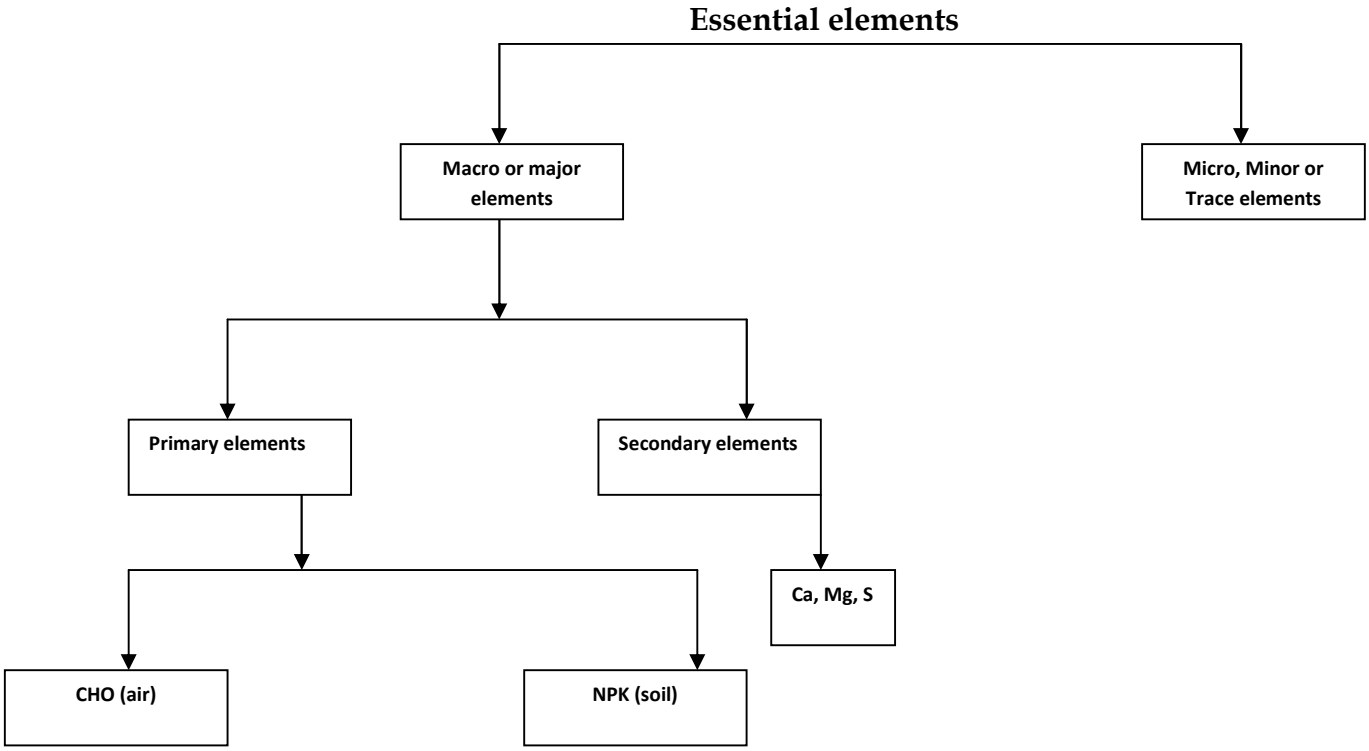
**Macro or major elements:** Elements required by plants in huge quantities i.e. 1000 mg/kg of dry matter. Examples – CHO NPK Ca Mg S**.**

**Micro, Minor or Trace elements:** Elements which are required by plants in less quantities i.e. 100 mg/kg of dry matter, Example Iron, Molybdenum, Copper, Chlorine, Boron, Manganese, Zinc and Nickel.

## Beneficial and other elements

Strontium, Selenium, Germanium, Fluorine, Gallium, Cobalt etc.

## Classification of elements




## Classification of elements according to their role and physiological functions (Mengel and Kirkby, 1987)

Plant nutrients have been divided into four basic groups.

1. The first group of essential elements form the organic (carbon) compounds of the plant. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction.
2. The second group is important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, borate and silicate esters in which the elemental group is bound to the hydroxyl group of an organic molecule (i. e. sugar- phosphate).

3. The third group is present in plant tissues as either free ions or ions bound to substances such as pectic acid present in the cell wall of particular importance or their roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group has important roles in reactions involving electron transfer.

## Classification of plant mineral nutrients according to biochemical functions

**Group 1:** This group includes nutrients that are part of organic compounds.

**Nitrogen:** Constituent of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexoamines etc.

**Sulphur:** Component of cysteine, methionine and proteins. Constituents of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, adenosine-5 phosphosulphate and 3 phospho adenosine.

**Group 2:** Nutrients that are important in energy storage or structural integrity.

**Phosphorus:** Component of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid etc. Has a role in reactions that involve ATP.

**Silicon:** Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties including rigidity and elasticity.

**Boron:** Forms complexes with mannitol, mannan, polymannuronic acid and other constituent of cell walls. Involved in cell elongation and nucleic acid metabolism.

**Group 3:** Nutrients that remain in ionic form.

**Potassium:** Required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electro neutrality.

**Calcium:** Constituent of middle lamella of cell walls. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a second messenger in metabolic regulation.

**Magnesium:** Required by many enzymes involved in phosphate transfer. Constituent of the chlorophyll molecule.

**Chlorine:** Required for the photosynthetic reactions involved in $O_2$ evolution.

**Manganese:** Required for activity of some dehydrogenase, decarboxylase, kinases, oxidases and peroxidases. Involved with other cation- activated enzymes and photosynthetic $O_2$ evolution.

**Sodium:** Involved in the regeneration of phospho enol pyruvate in $C_4$ and CAM plants . Substitute for potassium in some functions.

**Group 4:** Nutrients that are involved in redox reactions.

**Iron:** Constituent of cytochromes and nonheme iron proteins involved in photosynthesis, nitrogen fixation and respiration.

**Zinc:** Constituent of alcohal dehydrogenase, carbonic anhydrase etc.

**Copper:** Component of ascarboic acid oxidase, tyrosine, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase and plastocyanin.

**Nickel:** Constituent of urease. In nitrogen fixing bacteria constituent of hydrogenases.

**Molybdenum:** Constituent of nitrogenase, nitrate reductase and xanthine dehydrogenase.

## Essential elements for most higher plants and internal concentrations considered adequate

| Elements | Chemical symbol | Form available to plants | Atomic Wt. | Concentration in Dry Tissue | | Relative No. of Atoms Compared to Molybdenum |
|---|---|---|---|---|---|---|
| | | | | Mg/Kg | (%) | |
| Molybdenum | Mo | $MoO_4^{2-}$ | 95.95 | 0.1 | 0.00001 | 1 |
| Nickel | Ni | $Ni^{2+}$ | 58.71 | ? | ? | ? |
| Copper | Cu | $Cu^{+}$, $Cu^{2+}$ | 63.54 | 6 | 0.0006 | 100 |
| Zinc | Zn | $Zn^{2+}$ | 65.38 | 20 | 0.0020 | 300 |
| Manganese | Mn | $Mn^{2+}$ | 54.94 | 50 | 0.0050 | 1,000 |
| Boron | B | $H_3BO_3$ | 10.82 | 20 | 0.002 | 2,000 |
| Iron | Fe | $Fe^{3+}$, $Fe^{2+}$ | 55.85 | 100 | 0.010 | 2,000 |
| Chlorine | Cl | $Cl^{-}$ | 35.46 | 100 | 0.010 | 3,000 |
| Sulfur | S | $SO_4^{2-}$ | 32.07 | 1,000 | 0.1 | 30,000 |
| Phosphorus | P | $H_2PO_4^{-}$ $HPO_4^{2-}$ | 30.98 | 2,000 | 0.2 | 60,000 |
| Magnesium | Mg | $Mg^{2-}$ | 24.32 | 2,000 | 0.2 | 80,000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Calcium | Ca | $Ca^{2+}$ | 40.08 | 5,000 | 0.5 | 125,000 |
| Potassium | K | $K^{+}$ | 39.10 | 10,000 | 1.0 | 250,000 |
| Nitrogen | N | $NO_3^-$, $NH_4^+$ | 14.01 | 15,000 | 1.5 | 1,000,000 |
| Oxygen | O | $O_2$, $H_2O$ | 16.00 | 450,000 | 45 | 30,000,000 |
| Carbon | C | $CO_2$ | 12.01 | 450,000 | 45 | 35,000,000 |
| Hydrogen | H | $H_2O$ | 1.01 | 60,000 | 6 | 60,000,000 |

## Method of detection

## Ash Analysis

To detect some mineral elements of a plant, the plant materials are subjected to high temperatures (about $600^0$C) and then analyze its ash content. In the ash only the mineral elements are present, all of the organic compounds have been decomposed and passed of in the form of gases. The primary elements (carbon, hydrogen and oxygen) are given off as $CO_2$, water vapour and oxygen. Accurate determination of elemental nitrogen through this method is not possible as some part of it is given off in the form of ammonium or nitrogen gas. The elements in the ash are not present in their pure state but rather in the form of oxides. The qualitative and quantitative analysis of the ash for the different elements present is dependent on various chemical treatments. The chance of cumulative erroneous results gathered from these treatments is too great to allow heavy reliance on quantitative data for the majority of minerals obtained from the ash

**Table - Ash analysis of pride of Saline corn plants grown at Manhattan, Kansas**

Source- From Plant Physiology by E. C. Millar

| **Element** | **Weight ( g )** | **Total Dry Weight ( % )** |
|---|---|---|
| Nitrogen | 12.2 | 1.459 |
| Phosphorus | 1.7 | 0.203 |
| Potassium | 7.7 | 0.921 |
| Calcium | 1.9 | 0.227 |
| Magnesium | 1.5 | 0.179 |
| Sulphur | 1.4 | 0.167 |
| Iron | 0.7 | 0.083 |
| Silicon | 9.8 | 1.172 |
| Aluminum | 0.9 | 0.107 |
| Chlorine | 1.2 | 0.143 |
| Manganese | 0.3 | 0.035 |
| Undetermined elements | 7.8 | 0.933 |

Analysis of plant tissue. Finally, we must emphasize that, although ash analysis provides information concerning the relative amounts of minerals present in or taken up (e.g., aluminum and silicon) by the plant. These are not reliable methods for determining the extent of the utilization of these minerals by the plant.

## Physiological role of essential elements and their morphological and physiological deficiency symptoms

## Nitrogen

It is absorbed in the form of $NO_3^-$, $NO_2^-$ and $NH_4^+$.

**Functions**

It is very important due to its presence in protein molecule. Nitrogen is found in important molecules as purines, pyrimidines, porphyrins and cytochromes. Purines, pyrimidines are found in nucleic acids, RNA and DNA which are important for protein synthesis. The porphyrin structure is found in metabolically important compounds as the chlorophyll pigments and the cytochromes essential in photosynthesis and respiration. Coenzymes are essential to the function of many enzymes. Cytochromes act as electron carrier in photosynthesis and respiration processes.

**Deficiency symptoms**

Most important symptom is yellowing of leaves (chlorosis) due to loss of chlorophyll. The symptoms appear first in older leaves then in new or growing leaves due to its high mobility. The younger leaves retain their nitrogen and obtain N from older leaves as well through translocation. Under severe conditions of nitrogen deficiency the lowermost leaves on plants like in tobacco or beans dries. It also indirectly involved in anthocyanin pigment synthesis besides chlorophyll. In tomato due to nitrogen deficiency purple colour in leaf petioles and veins was noticed. If a plant is supplied higher concentration of N cell size and number increased due to increase in protein synthesis with an overall increase in leaf production (Morton and

Watson 1948; Neish 1957). Lutman (1934) noted a decrease in leaf epidermal size due to N deficiency in millet and buckwheat.

## Phosphorus

It is absorbed as $H_2\overline{P}O_4$ and $H\overline{P}O_4$ form. Low pH favours $H_2\overline{P}O_4$ and vice versa.

**Functions**

It is constituent of nucleic acid, phospholipids, the coenzyme NAD & NADP, constituent of ATP and other high energy compounds. Meristematic cells show high P concentration where P is involved in nucleoprotein synthesis. It is also involved in activation of amino acids through ATP for the synthesis of protein moiety. Phospholipids along with protein are constituent of cell membranes. The coenzymes NAD & NADP are important in oxidation reduction reactions in which hydrogen transfer takes place which controls photosynthesis, respiration, carbohydrate metabolism and fatty acid synthesis. Due to deficiency of P maturity is often delayed.

**Deficiency symptoms**

Phosphorus deficiency may cause premature leaf fall and purple and red anthocyanin pigmentation. Its deficiency causes development of necrotic areas on the leaves, petioles or fruits. The parts become stunted in appearance, leaves may have dark to blue green colouration. Older leaves show the deficiency symptoms first. Symptoms of zinc and phosphorus deficiencies may sometimes look alike for example, lack of either one of these elements may cause distortion in shape of leaves of some plants (Hewitt, 1963). Lyon and Garcia (1944) found increase in pith size with decreased vascular tissues during anatomical studies in tomato. Central pith cells had disintegrated and remaining cells were large and thin walled with abnormally large intercellular spaces. Phloem and xylem were thin walled with least development of these cells.

## Potassium

It is absorbed in $K^+$ form.

**Functions**

Deficiency affects processes like respiration, photosynthesis, chlorophyll development and water content of leaves. The important function of potassium is opening and closing of stomata. It is found in abundance in meristematic region. It activates the enzymes involved in the formation of certain peptide bonds. Accumulation of carbohydrates was often observed during the early stage of deficiency which is due to impaired protein synthesis (Eastin 1952). The carbon skeletons which normally go into protein synthesis are accumulated as carbohydrates. It activates several enzymes involved in carbohydrate metabolism. Apical dominance is lacking in K deficient plants.

**Deficiency symptoms**

Due to K deficiency chlorosis first occurs on the leaves followed by the development of necrotic areas at the tip and margin of leaf. The symptoms are first seen on the older leaves due to translocation of K into new leaves. There is a tendency for the leaf tip to curve downward in potato and French bean. Marginal regions may roll inward towards the upper surface. Stunted growth due to shortening of internode was also noticed in K deficient plants. In tomato K deficiency causes disintegration of pith cells which results in conversion of phloem parenchyma into sieve tubes and companion cells (Pfeffer 1990; Phillis and Mason 1940).

## Calcium

It is absorbed as $Ca^{++}$.

**Functions**

It is constituent of cell wall in the form of calcium pectate. The middle lamella is composed of calcium and magnesium pectates. Therefore, it is essential for formation of cell membranes and lipid structures. Calcium in small amounts is necessary for mitosis. Hewitt (1963) has suggested that calcium may be involved in chromatin or mitotic

spindle organization. Abnormal mitosis may develop because of an effect of calcium deficiency on chromosome structure and stability. It plays a role as an activator of enzyme phospholipase in cabbage leaves (Davidson and Long 1958). It also activates enzymes arginine kinase, adenosine triphosphates, adenyl kinase and potato apyrase (Mazia 1954). Florell (1956, 1957) found reduction in mitochondria number in wheat roots. In cotton deficiency results in increased levels of carbohydrates in the leaves and decreased levels in the stems and roots.

**Deficiency symptoms**

Due to deficiency of calcium meristematic regions of stem, leaf and root tips are greatly affected and die. Roots may become short, stubby and brown in calcium deficient plants (Kalra 1956). Chlorosis occurs along the margins of younger leaves, areas become necrotic. Malformation or distortion of the younger leaves was also noticed in calcium deficient plants. Hooking of leaf tip is also seen. Deficiency symptoms appear first in younger leaves and growing points due to immobility of calcium. Cell walls may become brittle or rigid (Davis 1949; Kalara 1956). Lutman (1934) observed vacuolation of cells in root apex of Calcium deficient rape and buckwheat plants.

## Magnesium

It is absorbed as $Mg^{++}$.

**Functions**

It is constituent of chlorophyll molecule without which photosynthesis would not occur. Magnesium acts as activator of enzymes involved in carbohydrate metabolism. It activates the enzymes involved in synthesis of nucleic acids (DNA, RNA) from nucleotide phosphates.

## Some enzymes involved in carbohydrate metabolism which require $Mg^{2+}$ as an activator

| Enzyme | Reactants | End products |
|---|---|---|

| | | | |
|---|---|---|---|
| Glucokinase | - | Glucose + ATP ⟶ | Glucose 6 phosphate |
| Fructokinase | - | Fructose + ATP ⟶ | Fructose 1 – P |
| Galactokinase | - | Galactose + ATP ⟶ | Galactose 1 – P |
| Hexokinase | - | Glyceraldehyde + ATP ⟶ | Phosphoglyceraldehyde |
| Gluconolactonase | - | 6 Phosphogluconolaction ⟶ | 6 Phosphogluconate |
| 6 Phosphogluconic dehydrogenase | - | 6 Phosphogluconate ⟶ | Ribulose – 5 – P |
| Enolase | - | 2 Phosphoglycerate +ATP ⟶ | Phosphoenol pyruvate |
| Pyruvic kinase | - | Phosphoenol pyruvate + ATP ⟶ | Pyruvate |
| Carboxylase | - | Pyruvate ⟶ | Acetaldehyde |
| Phosphoglyceric kinase | - | 1,3 Diphosphoglycerate+ ADP ⟶ | 3Phosphoglycerate |

**Deficiency symptoms**

It is constituent of chlorophyll, hence deficiency causes interveinal chlorosis in leaves. Initially yellowing is seen in the basal leaves, as the deficiency becomes more acute the yellowing is seen in new leaves also. Chlorosis is sometimes followed by the appearance of anthocyanin pigments in leaves. At more acute deficiency necrotic spots may be seen over leaves. Lyon and Garcia (1944) observed in tomato plants that excess supply of Mg caused a depression of internal phloem development and an increase in size of parenchymatous cells adjacent to endodermis. A deficient supply caused extensive chlorenchyma development with decrease in cell size though greater in number and densely packed with chloroplasts. Smaller pith cells were also observed under deficient conditions.

## Sulphur

It is absorbed as ($SO_4^{2-}$).

**Functions**

Its main function is its participation in protein structure in the form of sulphur bearing amino acids Viz; cystine, cysteine and methionine. It is taken up by the plants

as sulphate ($SO_4^{2-}$) which is later on reduced via an activation step involving the compound 3 phosphoadenosine – 5 – phosphosulphate (PAPS) and ATP. PAPS is synthesized in two steps 1. An activation of sulphate by ATP 2. The enzyme sulphurylase to form Adenosine 5 phosphosulphate (APS) followed by conversion of APS to PAPS by a specific kinase (Robbins and Lipmann 1956). S favours root formation. It is also necessary for chlorophyll formation.

$$SO_4^{2-} + ATP \xrightarrow[\text{Sulphurylase}]{Mg^{2+}} APS + P - P$$

$$APS + ATP \xrightarrow[\text{Kinase}]{Mg^{2+}} PAPS + ADP$$

The activated sulphate is eventually reduced and incorporated into cystine, cysteine and methionine and finally into protein structure. Sulphur is involved in the metabolic activities of vitamins Biotin, Thiamine and Coenzyme A. Sulphur forms cross links in the protein molecule and in conjunction with peptide and hydrogen bonding, acts to stabilize protein structure. It is a component of S adenosyl – methionine which is important in lignin and sterol biosynthesis. It is also important in Fe – S proteins in photosynthesis, N metabolism and ferredoxin synthesis.

**Deficiency symptoms**

Deficiency symptoms of S are similar to N deficiency in certain respects like in N deficient plants, there is a chlorosis in leaves followed by production of anthocyanin pigments in some species (Easton 1951). Unlike N deficient plants sulphur deficient plants show chlorosis on the younger leaves first. However, under severe conditions all leaves may be affected (Gilbert 1951). Hall and co-workers (1972) found sulphur deficiency results in decrease in stroma lamellae and increase in grana stakking in corn plants. Easton (1935, 1941, 1942 and 1951) found accumulation of starch, sucrose and

soluble nitrogen under deficient conditions in tomato, sun flower, black mustard and soybean but reducing sugars were lower than normal. He suggested that the increase in soluble nitrogen resulted from an inhibition of protein synthesis.

## Manganese

It is absorbed as $Mn^{++}$.

**Functions**

It acts as an activator of enzymes involved in the respiration and nitrogen metabolism. Enzymes of Krebs cycle, malic dehydrogenase and oxalo succinic decarboxylase requires the presence of manganese as an activator. Manganese acts as an activator for enzyme nitrate reductase and hydroxyl amine reductase (Nason 1956; Sadana and Mcelroy 1957). It is also involved in the destruction or oxidation of IAA (Goldacre 1961; Kenton 1955). Rate of photosynthesis decrease was also observed due to Mn deficiency in chlorella (Wiessner 1962). Mn is involved in electron transfer from water to chlorophyll during light reaction of photosynthesis.

**Deficiency symptoms**

Deficiency of $Mn^{++}$ is characterized by the appearance of chlorotic and necrotic spots on the interveinal areas of the leaves. Symptoms first appear on young leaves in some species, whereas in some species on older leaves. Hewitt (1945) and Piper (1942) noted brown necrosis in cotyledons of pea and bean seeds in Mn deficient plants. Eltinge (1941) found in tomato leaves that due to Mn deficiency chloroplasts lose chlorophyll and starch grains, become yellow green in colour, vacuolated and granular and finally disintegrate.

## Iron

It is taken up by the plants in the form of $Fe^{+++}$ (ferric) and $Fe^{++}$ (ferrous). The latter is metabolically more active.

**Functions**

Iron is directly incorporated into cytochromes as well as in compounds necessary for the electron transport in the mitochondria and into ferredoxin which is important for light reaction in photosynthesis. It is essential for chlorophyll synthesis. It is required in the synthesis of chloroplast proteins and enzymes involved in chlorophyll synthesis (Gauch and Duggar 1954). Price and Corell (1964) found increase in chlorophyll synthesis in Euglena cells with addition of iron. It is the component of various flavoproteins, metalloproteins involved in biological oxidations. It is also found in iron – porphyrin proteins, like cytochromes, peroxidases and catalases.

**Deficiency symptoms**

Important symptom is interveinal chlorosis in leaves. The younger leaves are most affected. More mature leaves show no chlorosis because of the immobility of iron in plants. Chlorosis sometimes followed by chlorosis of veins so that whole leaf becomes yellow. In severe cases the young leaves even become white with necrotic lesions. Lack of iron may inhibit formation of chloroplasts through inhibition of protein synthesis.

## Copper

Copper is absorbed as $Cu^{++}$.

**Functions**

It acts as component of phenolases, laccase, and ascorbic acid oxidase (Nason and Kaplan 1939). Grem and co-workers (1939) and Neish (1939) observed that copper is involved in the photosynthesis. Loustalot and others (1945) found that $CO_2$ absorption is decreased in copper deficient tung trees. The chloroplasts possess a copper containing protein called plastocyanin that is essential as an electron carrier in photosynthesis. Plastid enzymes namely phenolases contain copper that is essential to their functioning.

**Deficiency symptoms**

Deficiency brings Exanthema disease that is characterized by Gummosis (Gummy exudates) accompanied by dieback and glossy brownish blotches on leaves and fruits. Its deficiency also causes reclamation that is disease of cereals and characterized by chlorotic leaf tips and failure to set seeds. Copper deficiency causes a necrosis of tip of young leaves that proceeds along the margin of leaf and gives it a withered appearance. Under more severe conditions leaves may be lost and whole plant may appear wilted.

## Zinc

It is absorbed as $Zn^{++}$.

**Functions**

It is involved in biosynthesis of auxins. Skoog (1940) observed a decrease in auxin content in zinc deficient tomato plants. Scientists also concluded that zinc deficiency reduces auxin content through its involvement in the synthesis of tryptophan, a precursor of auxin (Tsui 1948). It participates in the metabolism of plants as an activator of several enzymes like carbonic anhydrase which converts carbonic acid into carbon di oxide and water. Other enzymes dependent on presence of zinc are alcohal dehydrogenase and pyridine nucleotide dehydrogenase (Hewitt et al. 1963; Nason et al. 1953) .Zn may also acts as an indicator of some phosphorus transferring enzymes, such as hexose kinase or triose phosphate dehydrogenase. Zn deficiency causes accumulation of soluble nitrogen compounds such as amino acids and amides (Possingham 1956).

**Deficiency symptoms**

The first sign of Zn deficiency is an interveinal chlorosis of older leaves starting at tips and margins, white necrotic spotting soon follows as in cotton (Brown and Wilson 1952). Leaves smaller, internode shortened resulted in stunted growth. Distorted appearance of leaves is also one of the deficiency symptoms. These are generally smaller in size, distorted in shape and appearance and may be clustered on short branches known as rosettes. This disease is referred as little leaf disease. Seed

production in beans and peas and development of fruit in citrus is also affected adversely in Zinc deficient plants.

## Boron

It is absorbed as $H_3BO_3$.

### Functions

Gauch and Duggar (1954) observed that boron is involved in carbohydrate transport within the plant. Uptake and translocation of sugar is retarded in Boron deficient plants. It also plays an important role in DNA synthesis in meristems. Important for cellular differentiation and development, nitrogen metabolism, fertilization, active salt absorption, hormone metabolism, water relations, fat metabolism and photosynthesis. It is involved indirectly through translocation of sugar.

### Deficiency symptoms

Death of root and shoot tip due to its requirement for DNA synthesis. Leaves may have thick coppery texture and some curl and become quite brittle. Flowers do not form and root growth is stunted. Disintegration of internal tissues results in abnormalities such as heart rot of sugarbeet, internal cork formation in apples, water core development in turnips, stem crack in celery, drought spot of apple .

## Molybdenum

It is absorbed as $MoO_4^{2-}$.

### Functions

It acts as catalyst in the reduction of nitrates. It is required for functioning of enzyme nitrate reductase which reduces nitrates to nitrites and subsequently to Ammonia. Its deficiency also causes drop in the concentration of ascorbic acid in the plant (Hewitt et al. 1950). It is also involved in phosphate metabolism. Lime increases its availability.

### Deficiency symptoms

Deficiency causes chlorotic interveinal mottling of leaves, followed by marginal necrosis and infolding of leaves. Under more severe conditions mottled areas may become necrotic and may cause leaf to wilt. Flower formation is inhibited, if forms then drops down before fruit setting. Its deficiency causes whip tail disease in cauliflower plants. The leaves first show interveinal mottling, margin becomes grey and flaccid and finally brown. The leaf tissue collapses leaving only mid rib and small pieces of leaf blade which appears as whip or tail.

## Chlorine

It is absorbed as $Cl^-$.

### Functions

It is necessary for photosynthesis. It acts as an activator of enzymes concerned with photolysis of water in which water splits up and $O_2$ is evolved. It also accelerates activation of amylase which converts starch into soluble sugars. It is essential for roots, for cell division in leaves and as an osmotically active solute (Terry 1977; Flowers 1988).

### Deficiency symptoms

$Cl^-$ deficiency causes reduced growth, wilting and development of chlorotic and necrotic spots. Leaves may attain a bronze colour. Roots become stunted in length but thickened or club shaped near the tip.

## Nickel

It is absorbed as $Ni^{++}$.

### Functions

It is part of enzyme urease which catalyses hydrolysis of urea to $CO_2$ and $NH_4^+$. In plants urea has the toxic effects and hydrolysis is necessary which is done by enzyme urease which contains nickel. It is also essential for germination of seeds (Brown et al. 1987).

**Deficiency symptomes**

Deficiency causes necrotic spots on leaves due to increase in ureides concentration in leaves.

## Metal toxicity and resistance

There is considerable genetic variation in the abilities of various species to tolerate otherwise toxic amounts of Cadmium, Silver, Mercury, Aluminum, Tin and other metals (Woolhouse 1983). In some species the elements are absorbed only to a limited extent, so this more accurately represents avoidance rather than tolerance (Taylor 1987). In other cases the elements accumulate in roots with little transport to shoots. In still others, both roots and shoots contain much higher amounts of such elements than nontolerant species or varieties could live with. This represents the true tolerance.

Recently, an important and phytogenetically widespread mechanism of tolerance was discovered (Reviewed by Gekeler et al. 1989; Steffens 1990 and Rauser 1990). Metals are detoxicated by chelation with phytochelatins, small peptides rich in the sulphur containing amino acid cysteine. These peptides generally have two to eight cysteine amino acids in the centre of the molecule and a glutamic acid and a cysteine at opposite ends. The sulphur atoms of cysteine are almost certainly essential to bind the metals, but other atoms such as nitrogen or oxygen likely also participate.

Phytochelatins are produced in numerous species, but so far they have only been found when toxic amounts of a metal are present, so they can detoxify even essential metals. Their formation therefore represents a true adaptive response to an environmental stress. They act similarly to the far large metallothionein proteins that detoxify metals in humans and other animals, but in contrast phytochelatins do not represent direct gene products. Still genetic control of their production will no thought prove essential in understating how various species live on mine wastes and other soils.

Among various metals Silver, Mercury and Copper are the most toxic metals. Inorganic salts of the same metal may vary in their toxicity effects on microorganisms.

Thus copper as a cupric ammonium sulphate is more firmly bound by spores than in copper sulphate and silver iodide is less toxic than any other silver halides. Substances secreted by fungal spores eg. amino acids and hydroxy acids, form soluble chelate complexes with copper which then readily penetrate the spore. It has been observed that the organic mercurials are more toxic than inorganic ones in bacteria and fungi. This may be due to more effective uptake of organic mercuric compounds, although phenyl mercuric acetate is more toxic in the ionic form. The primary toxic action of metal cations is the formation of nonionized complexes with surface inorganic groups eg. phosphate, carboxyl and sulphydryl and that the different toxicities of the metals can be related with the varying strengths of surface binding. The hypothesis put forward to account for accumulation of metals in spores suggests that the entire spore protoplasm accumulates the metal so that it moves freely across the semipermeable barriers.

## 10 . LECTURE NOTES

**Hydroponics, aeroponincs, nutrient solutions, foliar spray and basal application of nutrients.**

## Hydroponics

It is the technique of growing plants with their roots immersed in nutrient solution without soil.

## Advantages

- Mineral salts can be provided in the desired requirements.
- By using distilled water in the nutrient solution contamination can be avoided.

## Technique of using

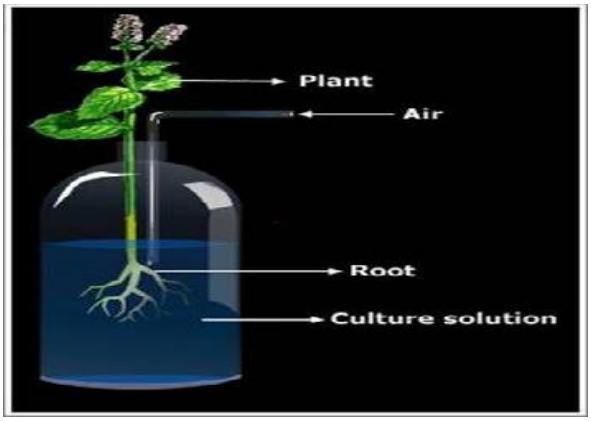
Several studies have shown that the best containers for solution cultures are made of borosilicate glass or natural polythene (Florell 1956). Still we can not say that these containers are contamination free due to presence of boron in borosilicate glass and molybdenum and cobalt in polythene. Water distilled in metal is also contaminated with trace amounts of copper, zinc and molybdenum.

In early studies of plant nutrition the nutrient reagent used presented a major source of contamination. These reagents had to be purified by various means before trace elements deficiencies could be demonstrated. Since most of the contamination is due to trace elements, study of major elements is not the serious problem as they are required in large quantities. One of the satisfactory methods of reversing the contamination is method of ridding water of contaminating trace elements is to pass it over cation and anion exchange resions (Florell, 1957). Next step is to prepare stock solution from inorganic salts containing necessary elements for normal plant growth.

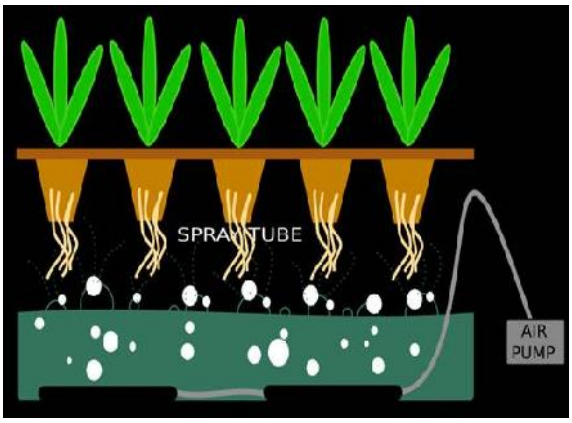
For studying the deficiency symptoms of a particular element that element should be left out of solution. In this technique the roots of the plant are submerged in the nutrient solution and stem projects through an opening cut in the container cover. For keeping

stem more tight padding material like cotton may be used. For obtaining good results aeration should be provided. Container needs to be covered to avoid the contamination due to atmospheric dust.

**Aeroponics:**- System in which roots are suspended over the nutrient solution, which is whipped into a mist by a motor driven rotor.




**Hydroponics**




**Aeroponics**

Nutrient solutions can be prepared by using the formulae given by various scientists as follows:

## Various nutrient solutions

| **Composition of some of the nutrient solutions** | | | |
|---|---|---|---|
| **Sach's Solution (1860)** | | **Knop's Solution (1865)** | |
| **Salts** | **Grams per litre** | **Salts** | **Grams per litre** |
| $KNO_3$ | 1.00 | $Ca(NO_3)_2$ | 0.8 |
| $Ca_3(PO_4)_2$ | 0.50 | $KNO_3$ | 0.2 |
| $MgSO_4.7H_2O$ | 0.50 | $KH_2PO_4$ | 0.2 |
| $CaSO_4$ | 0.50 | $MgSO_4.7H_2O$ | 0.2 |
| NaCl | 0.25 | $FeSO_4$ | 0.1 |
| $FeSO_4$ | Trace | | |
| **Hoagland's Solution modified by Arnon, 1940** | | | |
| **Salts** | **Grams per litre** | **Salts** | **Grams per litre** |
| $KNO_3$ | 1.02 | $CuSO_4.5H_2O$ | 0.08 |
| $Ca(NO_3)_2$ | 0.49 | $ZnSO_4.7H_2O$ | 0.22 |

| | | | |
|---|---|---|---|
| $NH_4H_2PO_4$ | 0.23 | $H_2MoO_4.H_2O$ (molybadic acid ) | 0.09 |
| $MgSO_4.7H_2O$ | 0.49 | $FeSO_4.7H_2O$ 0.5% | 0.6 ml/litre |
| $H_3BO_3$ | 2.86 | Tartaric acid 0.4% | |
| $MnCl_2.4H_2O$ | 1.81 | (3 x weekly) | |

**E.J. Hewitt and P. C. Steward (1963)**

| *(2) Salt* | *Gram / liter* | *ppm* | *mM / liter* |
|---|---|---|---|
| $KNO_3$ | 0.505 | K, 195; N, 70 | 5.0 |
| $Ca(NO_3)_2$ | 0.820 | Ca, 200; N, 140 | 5.0 |
| $NaH_2PO_4.2H_2O$ | 0.208 | P, 41 | 1.33 |
| $MgSO_4.7H_2O$ | 0.369 | Mg, 24 | 3.0 |
| Ferric nitrate | 0.0245 | Fe, 5.6 | 0.1 |
| $MnSO_4$ | 0.002230 | Mn, 0.550 | 0.01 |
| $CuSO_4.5H_2O$ | 0.000240 | Cu, 0.064 | 0.001 |
| $ZnSO_4.7H_2O$ | 0.000296 | Zn, 0.065 | 0.001 |
| $H_3BO_3$ | 0.001860 | B, 0.370 | 0.033 |
| $(NH_4)_6Mo_7O_{24}.H_2O$ | 0.000035 | Mo, 0.019 | 0.0002 |
| $CoSO_4.7H_2O$ | 0.000028 | Co, 0.006 | 0.0001 |
| NaCl | 0.005850 | Cl, 3.550 | 0.1 |

## Solid medium culture

Through solid medium such as sand, crushed quartz or gravel is easier to work than liquid medium but purification problem do exists. However, we can obtain purified silica and crushed quartz that are low in trace elements.

In this technique nutrient solutions are added into the solid culture. This is done in three ways: By pouring nutrient solution over the surface (slop culture), by dripping over the surface (drip culture) and by forcing solution up from the bottom of the container by using pumping apparatus (sub-irrigation system).In all three systems the

excess solution should be drained out through an opening at the bottom of the container.

## Foliar or aerial spray

Technique of spraying the mineral nutrients and other substances over the leaves.

## Advantages

- Insecticides, pesticides, herbicides, fungicides and growth regulating substances can be sprayed by this technique.
- In dry weather aerial spray is better than soil application.
- Leaching losses can be avoided.
- Requirement of nutrient or mineral is less.
- Quick absorption of nutrients and elements by plants.
- More economical than soil application.
- Certain elements like Mn, Zn, Cu and Fe gets precipitated in alkaline soil. By foliar application they can be easily made available.
- Availability of fertilizers to deep rooted crops is little late and this can be overcome by foliar spray.

## 11 . LECTURE NOTES

### Outer space and apparent free space, theories of active and passive absorption viz; Donnan's equilibrium, contact exchange, carrier concept, ion-exchange or cytochrome pump theory.

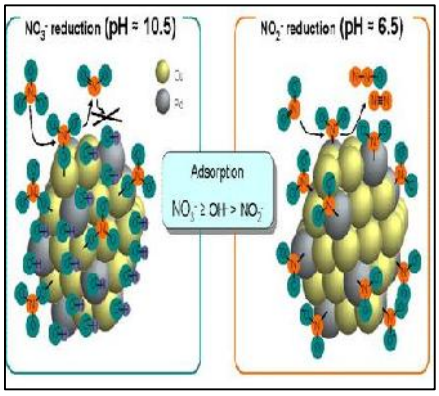
Certain terms are associated with the uptake of substances.

**Sorption:** When a molecule, ion or atom come in contact with some surface.

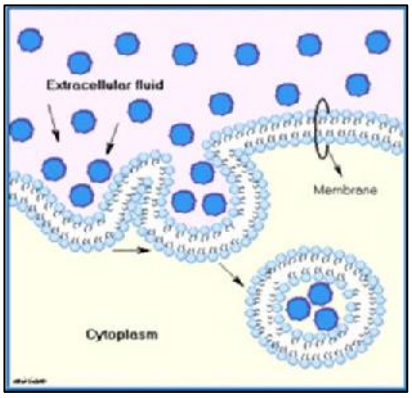
**Divided into two parts**

**Adsorption:** Binding of ions or molecules to a surface (e.g., of a soil particle or a root).

**Absorption:** When a molecule, ion or atom enters inside the cell.




**Adsorption**




**Absorption**

**Absorption- It can be divided in two parts:**

1. **Active absorption:** Absorption process which involves metabolic energy. The movement of substances may occur against or up gradient or chemical potential. Sometimes anions and cations accumulate against the concentration gradients which does not include the Donnan's effect. Ion transport requires metabolic energy. Ion accumulation is retarded due to decrease in metabolic energy which may be due to low temperature, low $O_2$ tension, metabolic inhibitors etc.

1. **Passive absorption:** The spontaneous downhill movement of molecules or ions without involvement of metabolic energy.

**Passive absorption can be divided in two parts**

1. **Diffusion**: Random movement of ions, molecules or atoms from area of higher concentration to lower concentration or from area of high kinetic energy to low kinetic energy influenced by kinetic energies of diffusing molecules.
2. **Mass flow:** Movement of ions, molecules or atoms in mass due to transpirational stream or pull**.**

Some scientists believe that ions can move inside roots along with the mass flow of water due to transpirational pull. According to this theory an increase in transpiration increases the absorption of ions (Russel and Barber 1960). Lopushinsky (1964) noted in the experiments with tomato using radioactive isotopes 32 P and 45 Ca that increase in absorption increases the salt absorption. Accumulation of ions against a concentration gradient is possible under mass flow mechanism due to an ion exchange mechanism or Donnan effect and equilibrium. The mass flow of ions through root tissue may also be possible with the aid of transpirational pull.

## Outer space and apparent free space

The outer space is defined as part of plant cell or tissue which allows free diffusion to take place, whereas apparent free space is the apparent volume of plant tissue for accommodating the diffusion of ions freely.

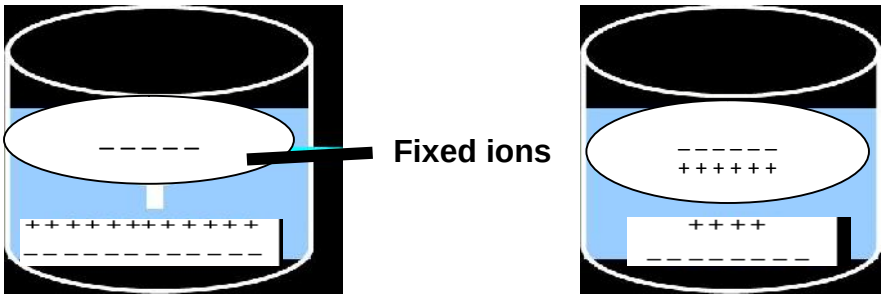
Salt absorption takes place through the intimate contact of the root system with the soil colloids or soil solution. Investigations have shown that ions are also absorbed through passive process also called nonmetabolic absorption. It has been observed that when a plant cell or tissue is transferred from a medium of low salt concentration to a medium of high salt concentration, there is an initial rapid uptake of ions, followed by a slow steady uptake that is under metabolic control. The initial rapid uptake is not affected by temperature or metabolic inhibitors indicates noninvolvement of metabolic energy. If the above tissue is returned to the low salt volume, some of the ions taken up will diffuse out into the external medium. In other words, a part of cell or tissue immersed in the salt solution is opened to free diffusion of ions. Since free diffusion implies that ions can move freely in or out of the tissue, the part of the tissue opened to

free diffusion will reach an equilibrium with the external medium and the ion concentration of this part will be the same as that found in the external medium. In response to the concept of outer space researchers turned to the task of calculating the volume of plant cell or tissue involved. They immersed a tissue in a solution of known concentration, allowed it to come to equilibrium and then determined the amount of salt taken up. Hope and Stevens (1952) found that bean root tips, when immersed in KCl solution, reached equilibrium in 20 minutes. The reversible diffusion of KCl took place in the absence of metabolic energy and the volume of tissue involved was considered to include a part of the cytoplasm. Hope (1953) demonstrated that the measured volume of the tissue allowing free diffusion increased when the concentration of KCl in the external solution is increased and since active transport was inhibited, it is assumed that a passive accumulation of ions against concentration gradient have occurred .The term apparent free space was introduced to describe the apparent volume accommodating the free diffusion of ions.


## Theories (Passive absorption)

## Donnan's equilibrium:

The absorption takes place in response to fixed ions.



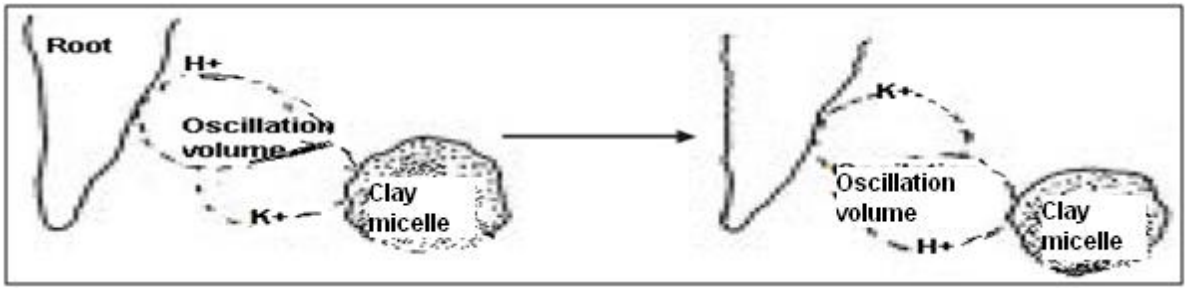

Suppose one cell is placed in the nutrient solution. Inside the cell membrane there is concentration of anions to which the membrane is impermeable. Suppose this membrane is permeable to anions and cations of the outer solution equal number of anions and cations will move across the membrane till equilibrium is reached. However, additional cations are needed to neutralize the fixed ions. Therefore, concentration of cations will be more inside the cell whereas, concentration of anions will be more in external solution. Therefore, ions can move inside the cell without involvement of energy against the concentration gradient in response to electrochemical potential gradient. When product of anions and cations in the internal solution is equal to that of anions and cations in the external solution the Donnan equilibrium is attained.

At equilibrium - $Ci^{+} Ai^{-} = CO^{+} AO^{-}$

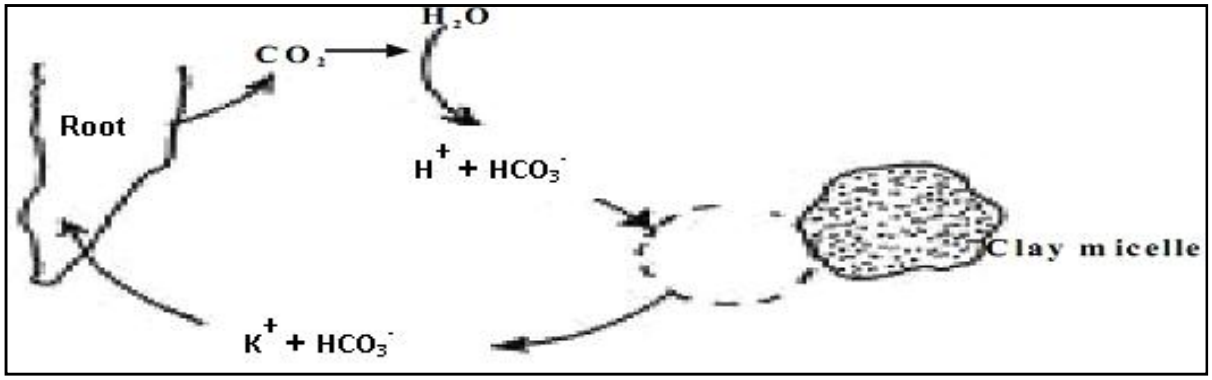
Ci = Cations Inside, Ai= Anions Inside

Co = Cations outside Ao = Anions outside

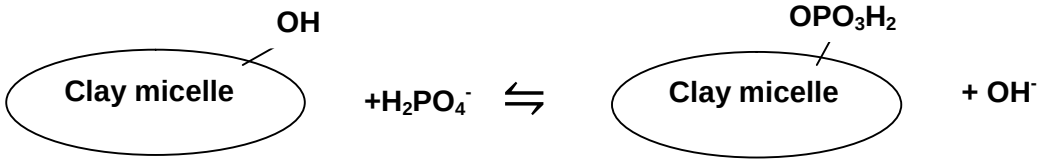
**Contact exchange theory:** Exchange of ions of the same charge held on the surface of soil colloids or root surface.



According to this theory (Jenny and Overstreet 1939) the ions adsorbed by the root surface or clay particles are not held very tightly but oscillates within certain volume of space, if two adsorbents are so close that oscillation volume of one ion overlaps oscillation volume of other ion, exchange takes place. Ions like $K^+$ are adsorbed on the surface of clay particles in the soil. These can be replaced if ions of same charge are made available. The $H^+$ ions held over the root surface are easily exchanged by the other ions of the same charge.




**Anion exchange:** Anion exchange may takes place between the minerals present in the micelles of soil and the phosphate ion. The anion $H_2PO_4^-$ replaces a hydroxyl anion from the surface of the clay micelle under mild acid conditions.




The addition of hydroxyl ions to the soil releasing the phosphate anion and raising the pH, thus also releasing phosphate from aluminum and ion complexes. However, over limiting which may cause a pH rise to over 7 could again tie up phosphate in the form of insoluble calcium phosphate.

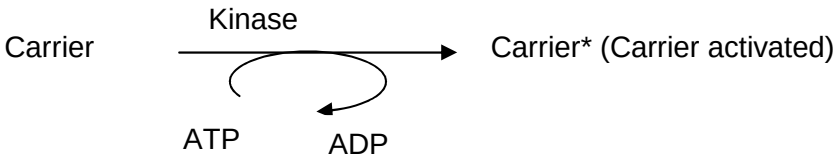
## Carbonic exchange theory

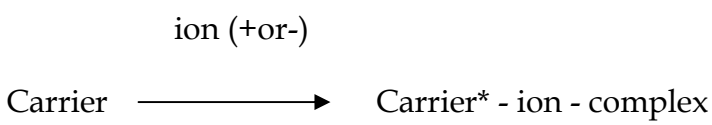
It is assumed that ions first dissolve in the soil solution, $CO_2$ released during the respiration dissolves in soil water forming carbonic acid ($H_2CO_3$) which is a week acid, it is converted into $H^+$ and $HCO_3^-$ ions. $H^+$ ions reach the clay particles and release other cations like $K^+$ from clay by exchange process. The released cations goes to the soil solution. Form the soil solution cations reach the root surface. The ion exchange does not require metabolic energy. Therefore, it is physical process.

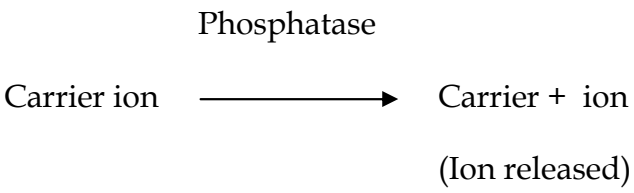
## Theories of active absorption

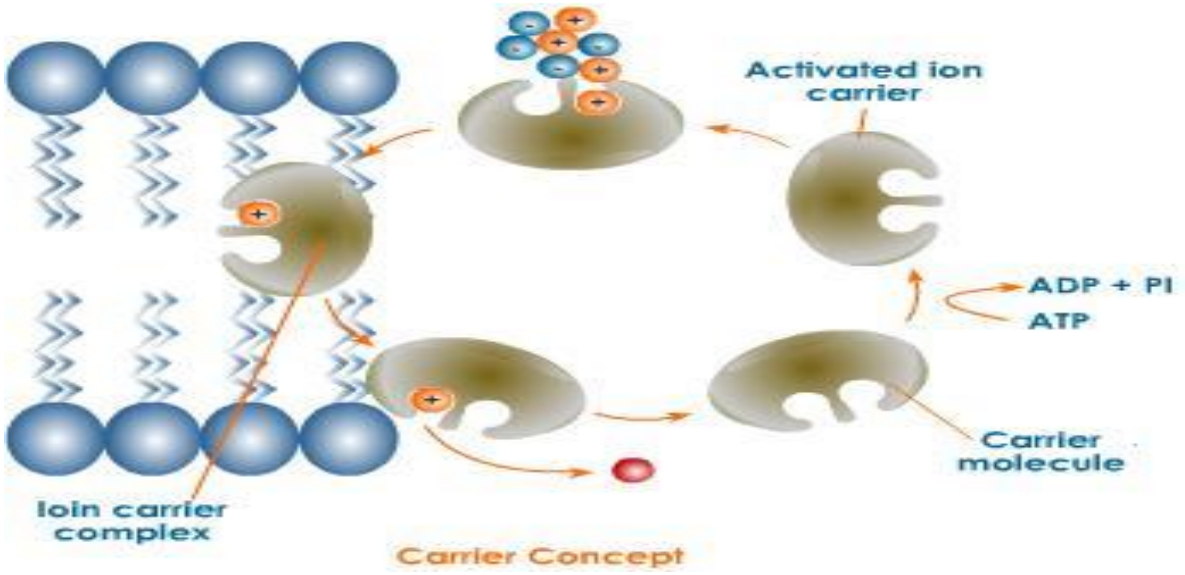
## Carrier hypothesis

It is believed that within the membrane there are some ion carriers. Outside the membrane ion combines with the carrier forming ion carrier complex. Now complex moves across membrane and reaches at the inner surface. Finally the complex is broken down on the inner face of the membrane through the action of phosphatase enzyme, the ion is released into the cytoplasm. The whole process requires the ATPs which are obtained through the respiration. The ATPs become available to the carrier by action of kinase enzyme, the process is called phosphorylation. In the process the ADPs are formed and carrier becomes activated reaches to the outer surface of the membrane and again gets ready to accept the other ion.






## Isotopic exchange

The carrier concept can be supported by using radioactive isotopes. Leggett and Epstein (1956) studied absorption of sulphate labeled with 35 S in barley excised roots. They observed that the total sulphate absorbed can be separated in two parts (i) Diffusible sulphate (ii) Actively absorbed $SO_4$.

## Saturation effects

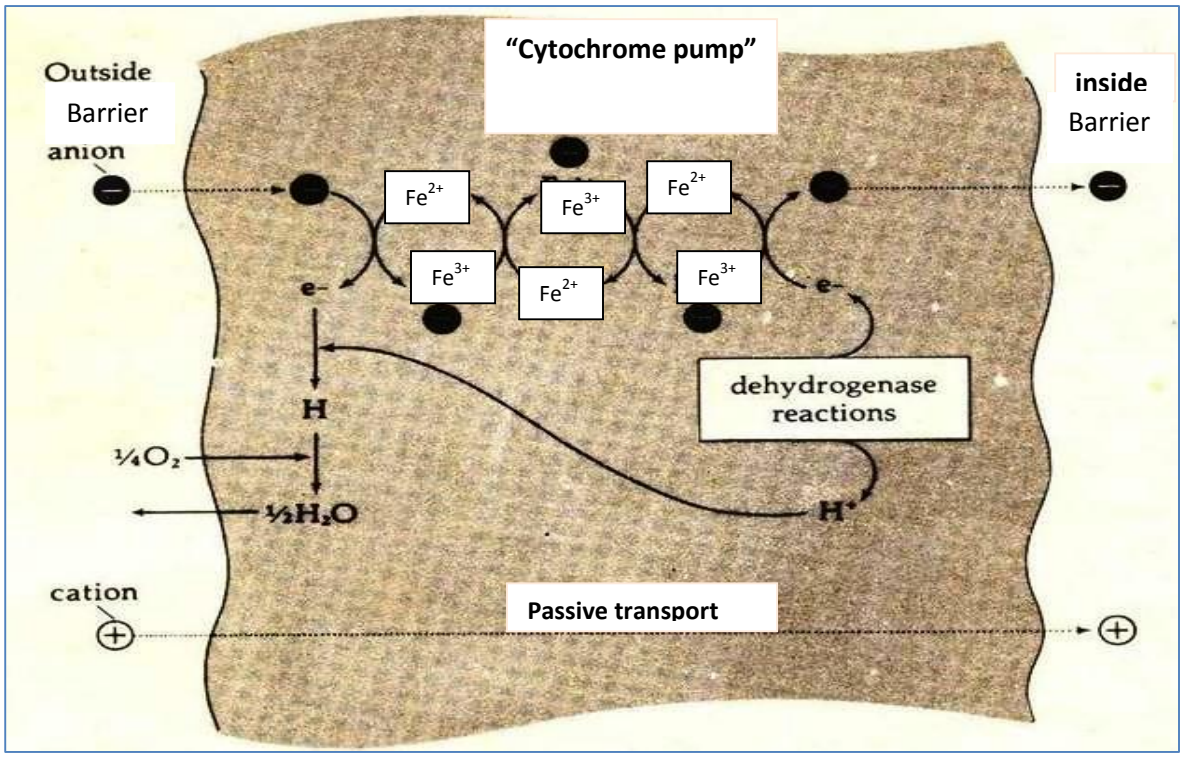
The concept presence of carriers in cell wall can also be demonstrated by experiments when there is a high salt concentration, absorption rate decreases due to engagement of all active sites or carriers.

## Specificity

Roots absorb ions selectively. There is a specific carrier for specific ions. Epstein and Hogen (1952) have shown that monovalent cations like Potassium, Cesium and Rubidium compete with each other for the same binding site. Absorption of one can be lowered by addition of $K^+$ or Cesium to the nutrient solution that can only be overcome by addition of rubidium.

## Ion pump mechanism: (Lundegardh and Burstrom 1933)

Ion absorption takes place through oxidation and reduction processes. Cation absorption occurs through passive process, whereas anion absorption takes place through cytochrome system.




Lundegardh and Burstrom (1933) claimed that there is close relationship between anion absorption and respiration. They observed that the rate of respiration increases when plant is transferred from water to salt solution. The increase of respiration rate due to transfer of plant tissue from water to salt solution is called salt respiration.

Later on Lundegardh (1950, 54) concluded:

1. Anion absorption is independent of cation absorption and takes place by different mechanism.

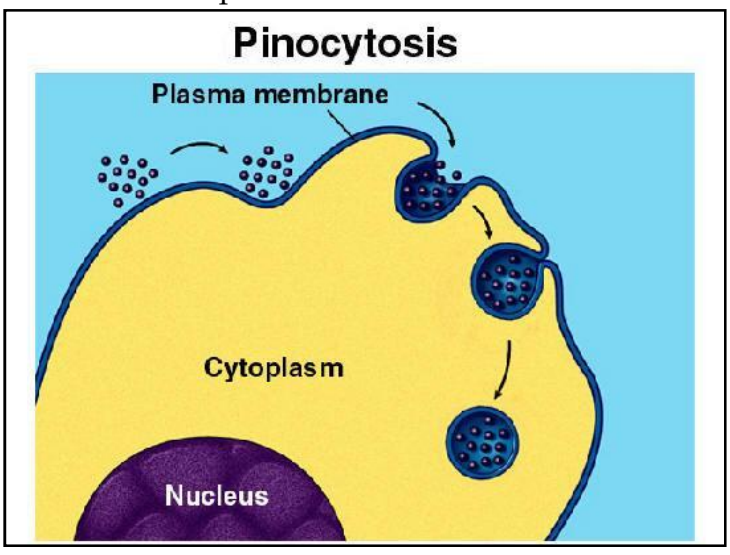
2. An oxygen gradient exists from the outer surface to the inner surface of the membrane which favours oxidation at the outer surface and reduction at the inner surface.
3. Actual transport of anion occurs through a cytochrome system.

According to Lundegardh's theory dehydrogenase reactions on the inner surface of the barrier or membrane produce protons ($H^+$) and electrons ($e^-$). The electrons move outward through cytochrome chain and anions move inward. At the outer surface of the barrier the reduced iron of the cytochrome is oxidized losing an electron and picking up an anion. The released electron unites with a proton and $O_2$ to form water. At the inner barrier surface the oxidised iron of cytochrome becomes reduced in dehydrogenase reactions. The anion is released on the inside of the barrier in the last reaction. Cations are absorbed passively to balance the potential difference caused by the accumulation of anions on the inner barrier surface.

## ATP Carrier Mechanism

Findings of Roberts, Wilkins and Weeks (1951) suggested that 2-4 dinitro phenol inhibited ATP formation resulting in decreased salt absorption. It clearly indicates participation of ATP in salt absorption.

**Pinocytosis:** It is the phenomenon which accounts for transport of larger molecules across the membrane like proteins, viruses etc. The plasma membrane is not smooth. The larger molecules first adhere to its surface. At this point the membrane inviginates and surrounds the particles on all sides forming a tiny vesicle or vacuole like structure around the particle. The vesicle is then pinched off from the membrane



and molecules are released into the cytoplasm. Here the vesicle membrane dissolves releasing the particle into the cytoplasm.

## 12 . LECTURE NOTES

### Membrane transporters, aquaporins, mechanism of ion or nutrient uptake and transport in plants, factors affecting nutrient uptake

## Membrane transporters

There are several transmembrane proteins facilitates the transport of molecules or ions across membrane as mentioned below:

## Channels

Transmembrane proteins that function as selective pores, through which molecules or ions can diffuse across membrane. Channels only permit passive absorption and limited mainly to ions or water. They have structures called gates which open and close the doors in response to external signals like voltage change, hormone binding, light etc.

## Pumps

Membrane proteins that carry out primary active transport across a biological membrane. Most pumps transport ions, such as $H^+$ or $Ca^{2+}$. ATP releases the energy when its terminal phosphate is hydrolysed. Reaction is catalyzed by ATP phosphohydrolase which is one of the transport proteins. This energy is used to transport protons ($H^+$) from one side of the membrane to other side against electrochemical gradient. This transport of $H^+$ provides energy that is used to transport essential mineral salts.

## Carriers

Proteins present in the membrane. During transport, the substances being transported is initially bound to a specific site on the carrier protein which was released free on the inner side of membrane enzymatically.

## Symporters

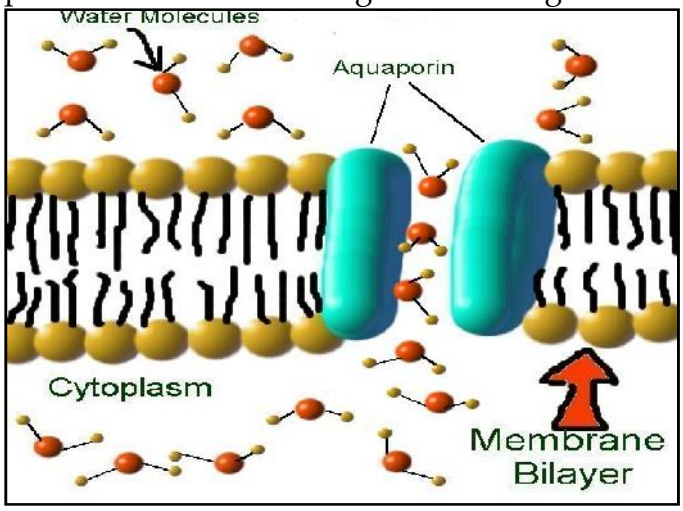
An integral membrane protein involved in movement of two or more different molecules or ions across a phospholipid membrane against the concentration gradient in the same direction. The phenomenon is called symport and proteins are called symporters.

## Antiporter

Coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction. The phenomenon is called antiport and the protein involved in the process is called antiporter.

## Aquaporins

These are class of proteins relatively abundant in plant membranes. Aquaporins reveal no currents when expressed in oocytes, but when the osmolarity of the external medium is reduced, expression of these proteins result in swelling and bursting of oocytes due to rapid influx of water across oocyte plasma membrane which normally has a low water permeability. Aquaporins form water channels in the membranes and the activity appears to be regulated by phosphorylation in response to water availability (Tyreman et al. 2002).




## Factor affecting salt absorption

## Temperature

An increase in temperature increases the salt absorption. However, beyond $40^0$C temperature there was a decrease in salt absorption which was mainly due to denaturation of enzymes involved in salt absorption. Temperature changes affect both passive and active absorption processes. The rate of free diffusion depends on kinetic energy of diffusing molecules which is dependant on temperature. Low temperature also reduces rate of biochemical reactions required for active transport.

**pH of soil:** The availability of ions in the soil solution is greatly affected by hydrogen ion concentration or pH of the soil. For example monovalent phosphate $H_2PO_4^-$ which is readily taken up by the plants is common in acidic soils. However, as soil approaches towards alkaline medium $HPO_4^{-2}$ and $PO_4^{-3}$ forms are available. $H_2PO_4^-$ form is easily taken up by the plants, $HPO_4^{-2}$ form is not easily taken up by the plant and $PO_4^{-3}$ form is not absorbed by the plants. Hence soils having low pH values are associated with higher absorption of phosphorus.

## Light

The effects of light on opening and closing of stomata and on photosynthesis indirectly affects salt uptake. Opened stomata increases mass flow of water which also accelerates salt absorption due to transpiration stream. The energy obtained from photosynthesis provides energy for active salt absorption and oxygen given off also improves conditions for active absorption of ions.

## $O_2$ tension

The salt absorption is retarded in absence of $O_2$ due to decrease in ion pump mechanism and oxidation reduction processes.

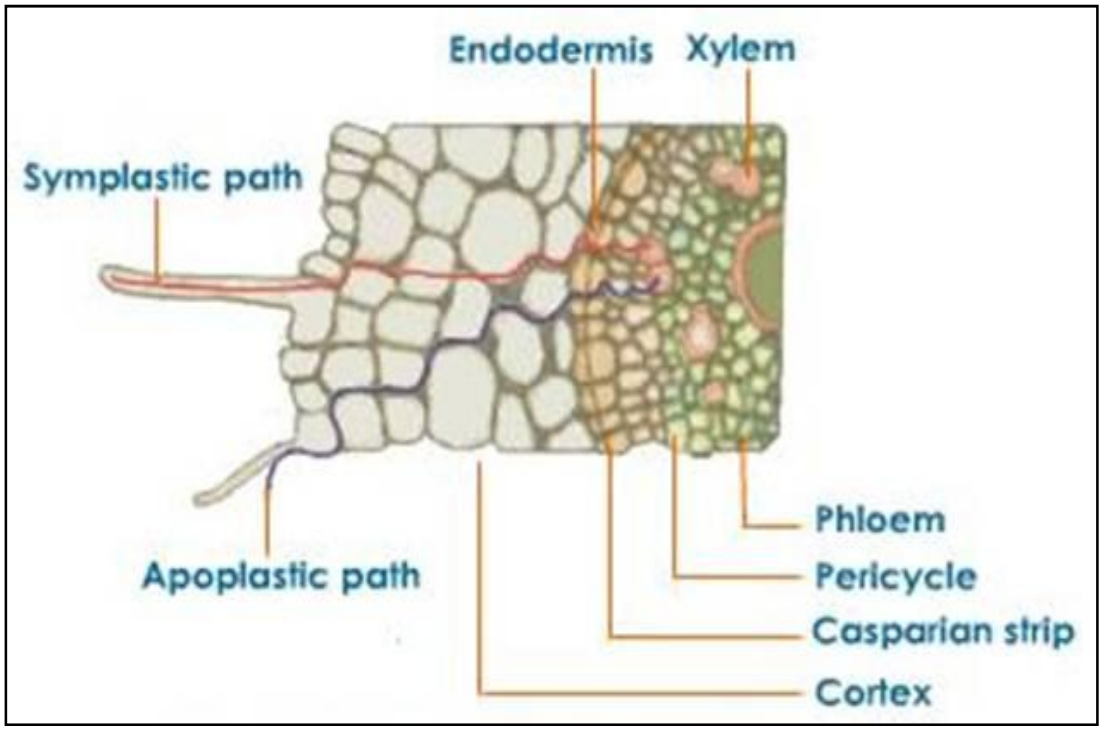
## Interaction of ions

Absorption of one element is affected by presence of others. Viets (1944) found that the $K^+$ absorption is affected by presence of $Ca^{++}$, $Mg^{++}$ and other polyvalent cations in external medium. He found that uptake of $K^+$ and bromine is less in absence of $Ca^{++}$, but it further decreases after the calcium concentration is increased past a maximum point. Olesen (1942) found that the absorption of $Mg^{++}$ is affected by presence of $Ca^{++}$. Competition for binding sites also affects salt absorption. Potassium, Rubidium and Cesium compete one another for mutual binding sites.

## Growth

The growth of plant increases surface area, number of cells, synthesis of new binding sites or carriers, factors etc. thereby increasing salt absorption. The increased volume of water taken up by a cell as it matures may dilute the internal concentration of salt and thus increase absorption activity. Stage of plant tissue also influences the salt absorption. Example – in roots with the age advancement suberin deposits over the roots which restricts further salt absorption. Rapid vegetative development demands elements and water which increases water movement and salt absorption through passive absorption.

## Mechanism of ion uptake




The actual absorption of salts by roots is both passive and active. The movement of salts into apparent free space is passive allowing for free diffusion of ions. Apparent free space may confined to cell walls and part of cytoplasm. The absorbed ions move freely up to endodermis where further penetration is retarded by casparian strip. Diffusing ions move unhindered through wet cell wall (apoplast) and plasmodesmata (symplast) of the cortex cells to the endodermis. Scientists have proposed various theories how passage of salts across endodermis takes place into xylem. Most accepted theory is a gradient of decreasing $O_2$ from cortex to stele (Crafts

and Broyer 1938). The living cells in the immediate area of xylem possess a low level of metabolic activity. Since energy is required to accumulate salt against a concentration gradient and to hold this salt, innermost cells favour the loss of salts. Thus it is thought that carrier system operates from cortex towards stele (Crafts 1951). Since diffusion back through the casparian strip is impossible there is unidirectional loss of salts into lumina of xylem vessels.

## Nutrient Uptake by Roots through Mycorrhizal Fungi

The absorption of mineral nutrients by roots may be modified by the association of mycorrhizal fungi with the root system. Mycorrhizae, singular mycorrhiza is originated from the Greek word for fungus and root. Much of the world's vegetation appears to have roots associated with mycorrhizal fungi. 83% of dicots, 79% of monocots, and all gymnosperms regularly form mycorrhizal associations (Wilcox 1991). Plants from the families Cruciferae (cabbage), Chenopodiaceae (spinach) and Proteaceae (macadamia nuts) as well as aquatic plants normally lacking mycorrhizae. They are absent from roots in very dry, saline, or flooded soils, or where soil fertility is extreme, either high or low. Plants grown under hydroponics and young, rapidly growing crop plants seldom have mycorrhizae.

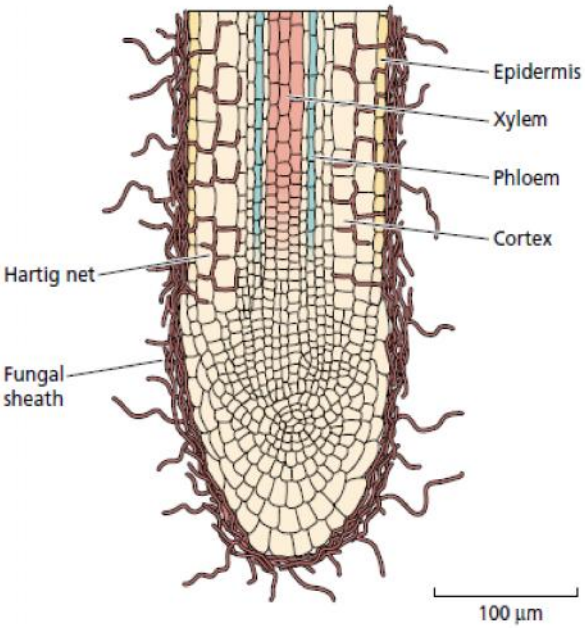
They are composed of fine tubular filaments called *hyphae* (singular *hypha*). Hyphae forming mass that normally forms the body of the fungus is called the *mycelium* (plural *mycelia*). There are two major classes of mycorrhizal fungi exists, ectotrophic and vesicular arbuscular mycorrhizae (Smith et al. 1997). Minor classes of mycorrhizal fungi include the ericaceous and orchidaceous mycorrhizae, which may have limited importance in terms of mineral nutrient uptake.

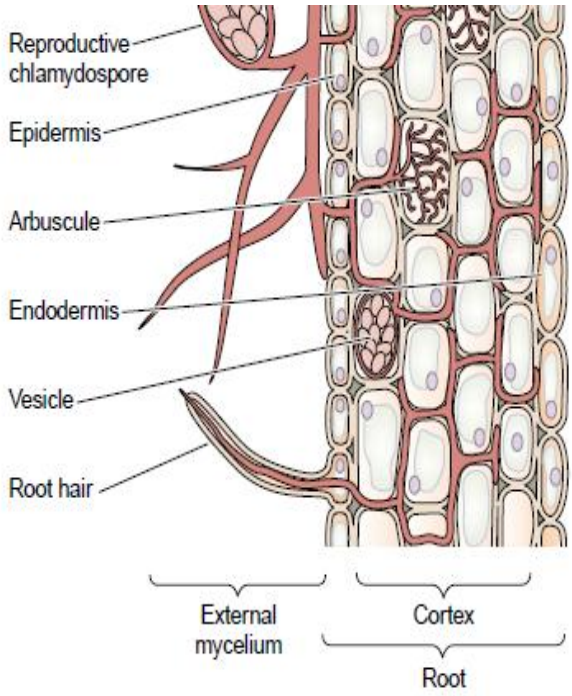
Ectotrophic mycorrhizal fungi typically show a thick sheath, or "mantle," of fungal mycelium around the roots, and some of the mycelium penetrates between the cortical cells which themselves are not penetrated by the fungal hyphae but instead are surrounded by a network of hyphae called the Hartig net. Often the amount of fungal mycelium is so extensive that its total mass is comparable to that of the roots

themselves. The fungal mycelium also extends into the soil, away from this compact mantle, where it forms individual hyphae or strands containing fruiting bodies. The capacity of the root system to absorb nutrients is improved by the presence of external fungal hyphae that are much finer than plant roots and can reach beyond the areas of nutrient-depleted soil near the roots (Clarkson 1985). Ectotrophic mycorrhizal fungi, vesicular-arbuscular mycorrhizal fungi do not produce a compact mantle of fungal mycelium around the root. Instead, the hyphae grow in a less dense arrangement, both within the root itself and extending outward from the root into the surrounding soil. After entering the root through either the epidermis or a root hair, the hyphae not only extend through the regions between cells but also penetrate individual cells of the cortex. Within the cells, the hyphae can form oval structures called vesicles and branched structures arbuscules which appear to be sites of nutrient transfer between the fungus and the host plant. Outside the root the external mycelium can extend several centimeters away from the root and may contain spore-bearing structures. Unlike the ectotrophic mycorrhizae, vesicular-arbuscular mycorrhizae make up only a small mass of fungal material, which is unlikely to exceed 10% of the root weight. Vesicular-arbuscular mycorrhizae are found in association with the roots of most species of herbaceous angiosperms (Smith et al. 1997). The association of vesicular-arbuscular mycorrhizae with plant roots facilitates the uptake of phosphorus and trace metals such as zinc and copper. By extending beyond the depletion zone for phosphorus around the root, the external mycelium improves phosphorus absorption. Calculations show that a root associated with mycorrhizal fungi can transport phosphate at a rate more than four times higher than that of a root not associated with mycorrhizae (Nye and Tinker 1977). The external mycelium of the ectotrophic mycorrhizae can also absorb phosphate and make it available to the plant. In addition, it has been suggested that ectotrophic mycorrhizae proliferate in the organic litter of the soil and hydrolyze organic phosphorus for transfer to the root (Smith et al. 1997).

## Nutrients Move from the Mycorrhizal Fungi to the Root Cells

Little is known about the mechanism by which the mineral nutrients absorbed by mycorrhizal fungi are transferred to the cells of plant roots. With ectotrophic mycorrhizae, inorganic phosphate may simply diffuse from the hyphae in the Hartig net and be absorbed by the root cortical cells. With vesicular-arbuscular mycorrhizae, the situation may be more complex. Nutrients may diffuse from intact arbuscules to root cortical cells. Alternatively, because some root arbuscules are continually degenerating while new ones are forming, degenerating arbuscules may release their internal contents to the host root cells. A key factor in the extent of mycorrhizal association with the plant root is the nutritional status of the host plant. Moderate deficiency of a nutrient such as phosphorus tends to promote infection, whereas plants with abundant nutrients tend to suppress mycorrhizal infection. Mycorrhizal association in well-fertilized soils may shift from a symbiotic relationship to a parasitic one in that the fungus still obtains carbohydrates from the host plant, but the host plant no longer benefits from improved nutrient uptake efficiency. Under such conditions, the host plant may treat mycorrhizal fungi as it does other pathogens (Brundrett 1991; Marschner 1995).




## Circulation of salts

Salts accumulated in the xylem ducts of the roots are translocated into the shoot and through shoots to various parts of the plant. There may be redistribution of elements like during abscission of leaves mineral salts are withdrawn from the leaf and redistributed to other parts of the plant. Generally circulation of elements takes place in the vascular tissues.

## Translocation of salts in the xylem

The salts accumulated in the xylem ducts of roots are carried upward with the transpiration stream. The salts move upward in the xylem tissues has been demonstrated in several ways. Experiments have shown that the upward translocation of salts is retarded if xylem is removed. It is thought that the salts move upward through the transpiration stream. Arnon, Stout and Sipos (1940) noted in the tomato plants that radioactive phosphate travelled upward to the tip of tomato plants more rapidly in transpiring plants. Sutcliffe (1962) showed that if transpiration by a leaf is inhibited by covering the leaf with a polythene bag it also inhibited salt absorption. Stout and Hoagland (1939) showed using radioactive isotopes that potassium is translocated upward in the xylem tissue. However, lateral interchange of potassium between xylem, cambium and phloem may takes place.

## Lateral translocation of salts

Generally xylem tissue is separated from the phloem tissue by a layer of cells called cambium. The cambial tissues regulate upward, lateral and downward movement of salts. Suppose if a particular element is present in phloem in high concentration, the cambium maintains balance by accommodating some salts inside. If a element is present in low concentration in phloem cambium enhances lateral translocation into the phloem.

## Translocation of salts into the phloem

It is thought that upward movement of salts takes place through xylem from where they are translocated into the phloem.

## Outward movement of salts from the leaves

Studies have shown that in deciduous plants prior to leaf abscission there is a movement of mineral nutrients out of leaves. Among nutrients moving out are N, P, K, S, Cl, Fe and Mg. Those remaining are Calcium, Boron, Manganese and Silicon. The withdrawal of mineral nutrients from leaves takes place in the phloem tissue.

## Circulation and reutilization

Mason and Maskell (1931-36) concluded that minerals are taken up through the transpiration stream and moved to the leaves and excess quantities are retranslocated downward into the phloem. The mineral salts could be laterally transported into the xylem tissue where upward translocation could take place again.

## 13 .LECTURE NOTES

### Mechanism of photosynthesis: light reaction, photolysis of water, quantum requirements and pigment systems, photophosphorylation (cycle and non cylclic).

**PHOTOSYNTHESIS**

- Process in which light energy is used to reduce $CO_2$ to organic compounds; occurs in chloroplasts in higher plants and algae.
- The conversion of light energy to chemical energy by photosynthetic pigments using water and $CO_2$ and producing carbohydrates.
- Process by which green plants manufacture complex carbonaceous substances from $CO_2$ and water in presence of solar energy and chlorophyll. $O_2$ being the end product.

$$6\,CO_2 + 12\,H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

**Raw materials for photosynthesis**

**Hydrogen Donor**

Water is the hydrogen donor. Water splits into $H^+$ & $OH^-$ ions. H is picked up by coenzyme NADP to form $NADPH_2$ which is used to reduce $CO_2$.

$4H_2O = 4H^+ + 4OH^-$, $4OH^- = 2H_2O + 4e^- + O_2$

$2NADP + 4H^+ = 2NADPH + 2H^+$

**Light**

Visible part of electromagnetic radiation occurs between the range of 390-760 mμ or nm (1 mμ or nm = $10^{-9}$ meter or $10^{-7}$ cm). Wave lengths shorter than 390 mμ or nm are called ultra-violet rays. Wave lengths longer than 760 – 100000 mμ are called infra-red rays. Beyond this are electric and radio waves which are measured in Kilometers.

**Photon**

- Individual particle of light.
- A discrete physical unit of radiant energy.

**Quantum (plural quanta)**

- A discrete packet of energy contained in a photon.
- Photon containing the energy.

**Energy constant**

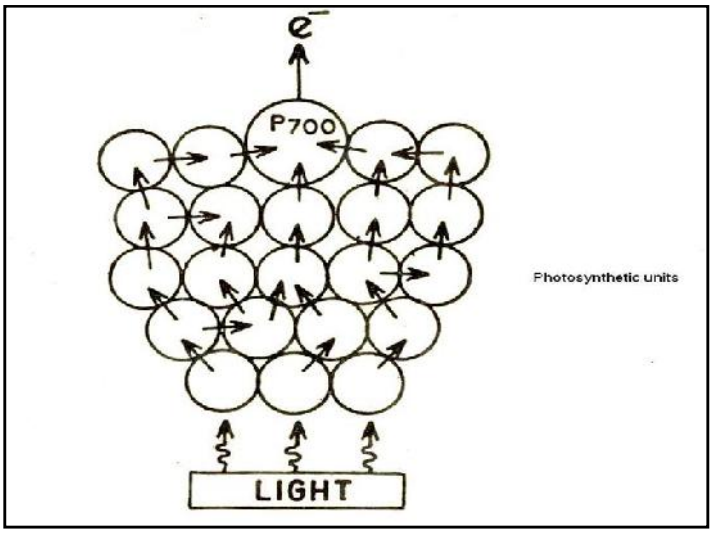
Magnitude of energy a photon contains is expressed as follows E= hc/ג

Where E = Energy constant, h= plank's constant (6.625 x $10^{-27}$ erg/sec).

C = velocity of light (3 x $10^{10}$ cm/sec). ג = Wave length size.

Shorter the wave length, higher will be the energy.

**Photosynthetic units or quantasomes**



Photosynthetic units

Smallest group of coordinating pigment molecules necessary to affect photochemical act i.e. absorption and transportation of light quantum to trapping centre where it causes excitation and release of an electron. They contain chlorophylls, carotenoids, quinones, lipids, glycerides and sterols.

Emerson and Arnold (1932) observed in chlorella that 2500 molecules of chlorophyll pigment are necessary to fix one molecule of $CO_2$. They termed this number 2500 as photosynthetic unit. The quantasomes are $180^0$ A broad and $100^0$A thick (18x16x10 nm). Each quantasome has 230 molecules of chlorophyll (160 chlorophyll a and 70 chlorophyll b), 48 carotenoids, 46 quinone compounds, 116 phospholipids, 144 digalactosyl diglycerides, 346 monogalactosyl glycerides, 48 sulpholipids, unidentified lipids and many sterols. Quantasomes have a molecular weight of about two million. The middle region of quantasome is called reaction centre.

**Photosynthetic apparatus**

They can be divided as Chloroplast – contains chlorophyll. Chromoplast –these are pigmented plastids contain carotenoids (xanthophyll, fucoxanthin). Leucoplasts- they contain starch called amyloplasts, oil containing- elaioplast, protein containing – aleuroneplast.

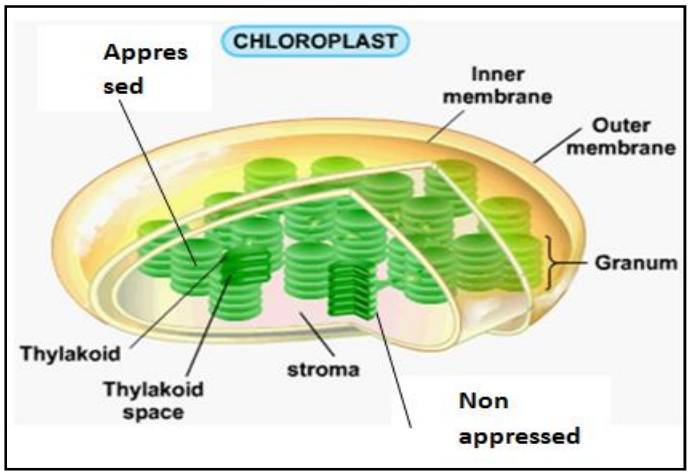
**Chloroplast**

- Membranes contain chlorophyll and its associated proteins, sites of photosynthesis.
- The organelle that is the site of photosynthesis in eukaryotic photosynthetic organisms.
- Spherical structures contain carbohydrates, proteins, lipids, chlorophylls and carotenoids. Light and dark reactions occur in grana and stroma, respectively.

**Structure of chloroplast**

They are generally spherical in shape, 1mμ thick and 4-6 mμ in length. No. varies from cell to cell. Chlamydomonas contains only one chloroplast. They contain carbohydrates, proteins, lipids, chlorophylls and carotenoids etc. It is covered with two membranes which are smooth and semipermeable. Inside is a clear structureless stroma contains enzymes that converts $CO_2$ into carbohydrates (dark reaction), starch grains and osmophilic droplets.

**Internal structure of a chloroplast granum**




Inside stroma system of membranes exit which run parallel to one another along the length of chloroplasts. They are called lamellae. They occur in pairs. Comparatively thin membranes are called stroma lamellae. In certain places 10-100 paired lamellae form disc type of structures and arranged one above the other like stack of coins called grana lamellae. The complete structure may be called as grana, whereas individual lamellae is called granum. They are also called granum thylakoid (Thylakoid- Greek word Thylacos (sac or pouch) as the ends or margins of paired lamellae are joined together to form closed disc shaped sacs called thylakoids. Chloroplast pigments occur in grana. In thylakoids light energy is used to oxidize water and form ATP and NADPH. The region where one granum thylakoid contacts another is called appressed region. Other region is called nonappressed region. They carry out

different photochemical reactions. Lamellae composed of proteins and phospholipids. Chloroplast consists of 40-50% proteins, 23-25% phospholipids, chlorophylls 5-10%, carotenoids 1-2%, RNA 5%, DNA in small amount. PS I is located towards stroma side of thylakoid. PS II and cyto $b_6$ f complex are found in appressed region, whereas PS I and cyto f are found in nonappressed region. Cyto $b_6$ is also called b 563. Cyto f contains iron.

**Arrangement of pigments in granum**

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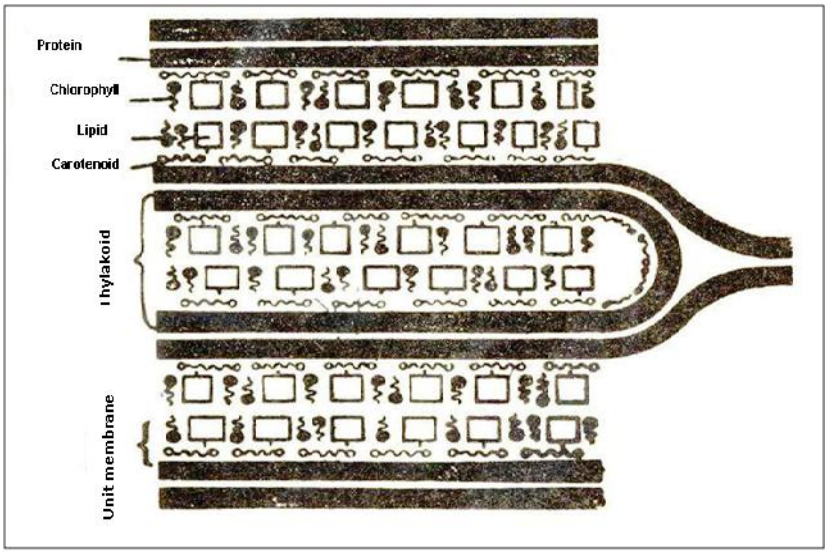



The chloroplast pigments occur in grana and are associated with thylakoid membranes. Each membrane is made up of a monomolecular protein layer to which chlorophyll molecules are attached. Inside this is a lipid layer containing the carotenoid molecules. The thylakoid is thus made up of two protein layers and space between them is occupied by two layers of lipids and molecules of chloroplast pigments. The lypoprotein membranes (sub units) between two thylakoids are appressed to each other forming partitions between successive thylakoids.

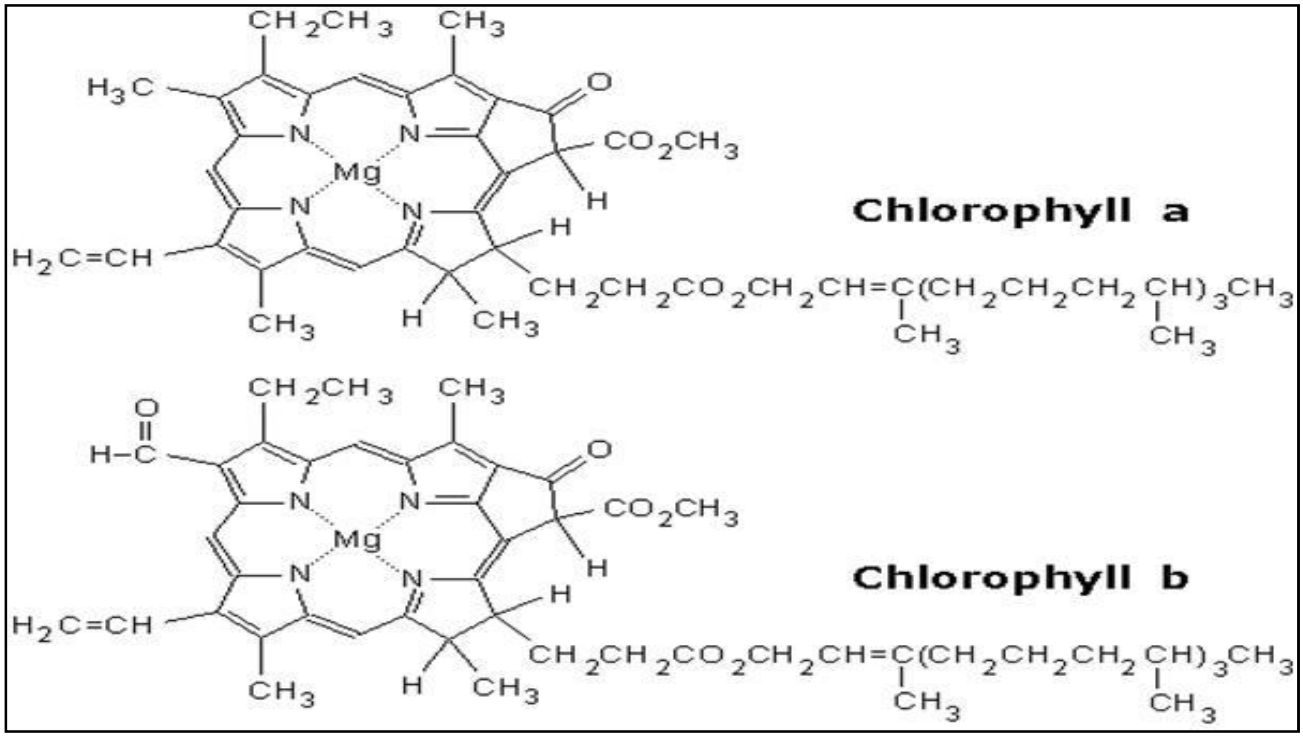
**Pigments in chloroplast**

**Chlorophylls**

These are pigments associated with light absorption during photosynthesis. Nine types have been identified viz., Chloro. a, b, c, d, e, bacteriochlorophyll a & b and chlorobium chloro. 650 and 660 mμ.

Chlorophyll a & b are found in green plants, whereas c, d and e are common in algae in combination with chlorophyll a. Bacterio-chlorophylls and chlorobium chlorophylls are found only in photosynthetic bacteria.

**Structure of chlorophyll molecule**




Chlorophyll molecule consists of a head and tail resembling a tennis racquet. The head is a porphyrin structure made up of four pyrrole rings attached to each other at the centre by an isocyclic ring containing Mg atom at the center. Extending from one of the pyrrole rings is the tail – the alcoholic chain (Phytol). They have different absorption spectra.

Empirical formula Chlo. a – $C_{55} H_{72} 0_5 N_4 Mg$

Chlo. b – $C_{55} H_{70} O_6 N_4 Mg$

The phytol chain is estrified on the C atom of one of the pyrrole rings has only one duoble bond . In chlorophyll a $C_3$ atom has a methyl group , while chlorophyll b has an aldehyde group.The peaks are as follows :

Chlorophyll a – 410, 429 and 660 nm.

Chlorophyll b – 430, 442 and 453.

**Biosynthesis**

Succinyl CoA which is an intermediate product in Kreb's cycle together with amino acid glycine initiates synthesis of chlorophyll.

**Carotenoids**

These are lipid compounds ranging in colour from yellow to purple, found in animals and plants both. These are also present in microorganisms including red algae, cyanobacteria, photosynthetic bacteria, fungi etc. Main carotenoid is Beta carotene found in plants possess orange yellow colour. Carotenoids and chlorophylls may be combined with same protein to form a complex known as photosynthein. Carotenoids are associated with transfer of light energy to chlo. a and protection of chlorophyll against photooxiation by forming epoxy ring.

**Phycobilins**

They are of two types Phycoerythrin (red) and Phycocyanin (blue) found only in algae. They are also involved in transfer of energy to chlorophyll. Phycoerythrin has peaks at 495, 540 and 545 nm and Phycocyanin has peaks at 550 and 615 nm.

**Mechanism of photosynthesis**

It is an oxidation and reduction process in which water is oxidized to $H^+$ and $OH^-$ and $CO_2$ is reduced to carbohydrate with water and $O_2$ being by products. During light reaction the energy necessary for reduction of $CO_2$ is produced, while in dark $CO_2$ is reduced to carbohydrates utilizing the energy produced in light reaction. Light reaction is also called photochemical decomposition of water and dark reaction is called thermochemical reduction of $CO_2$.

**Light reaction**

Light reaction takes place in grana and takes about $10^9$ Seconds, within such a short period of time there will be synthesis of molecules of ATP and NADPH which are utilized for dark fixation of $CO_2$.

**The following steps are covered in light reaction:**

**Photolysis of water or hill reaction**

Splitting of water in presence of light to produce $H^+$ and $OH^-$ ions is called photolysis of water. $H^+$ ions are used to reduce $CO_2$ and $OH^-$ ions recombine to form water along with release $O_2$ and $e^-$.

$4H_2 0 = 4 H^+ + 4 OH^-, 4 OH^- = 2 H_2O + 4 e^- + O_2$

These $4H^+$ are used to reduce $CO_2$.

$CO_2 + 4H^+ = CH_2O + H_2O$

**Source of $O_2$**

The work of Ruben, Kemen and Randall (1941) using isotope of $O_2$ ($O^{18}$) clearly showed that $O_2$ comes from water not from $CO_2$. When experimental material was supplied with labelled water ($H_2O^{18}$), the released $O_2$ was of $O^{18}$ type and when plant was supplied with labelled $CO_2^{18}$, the $O_2$ released was of normal type.

Bacteria use $H_2S$ as hydrogen donor in place of water.

$CO_2 + 2H_2S = CH_2O + H_2O + 2S$

**Arnons work**

Arnon showed that $CO_2$ fixation takes place in stroma while ATP and NADPH are produced in grana.

**Quantum requirements**

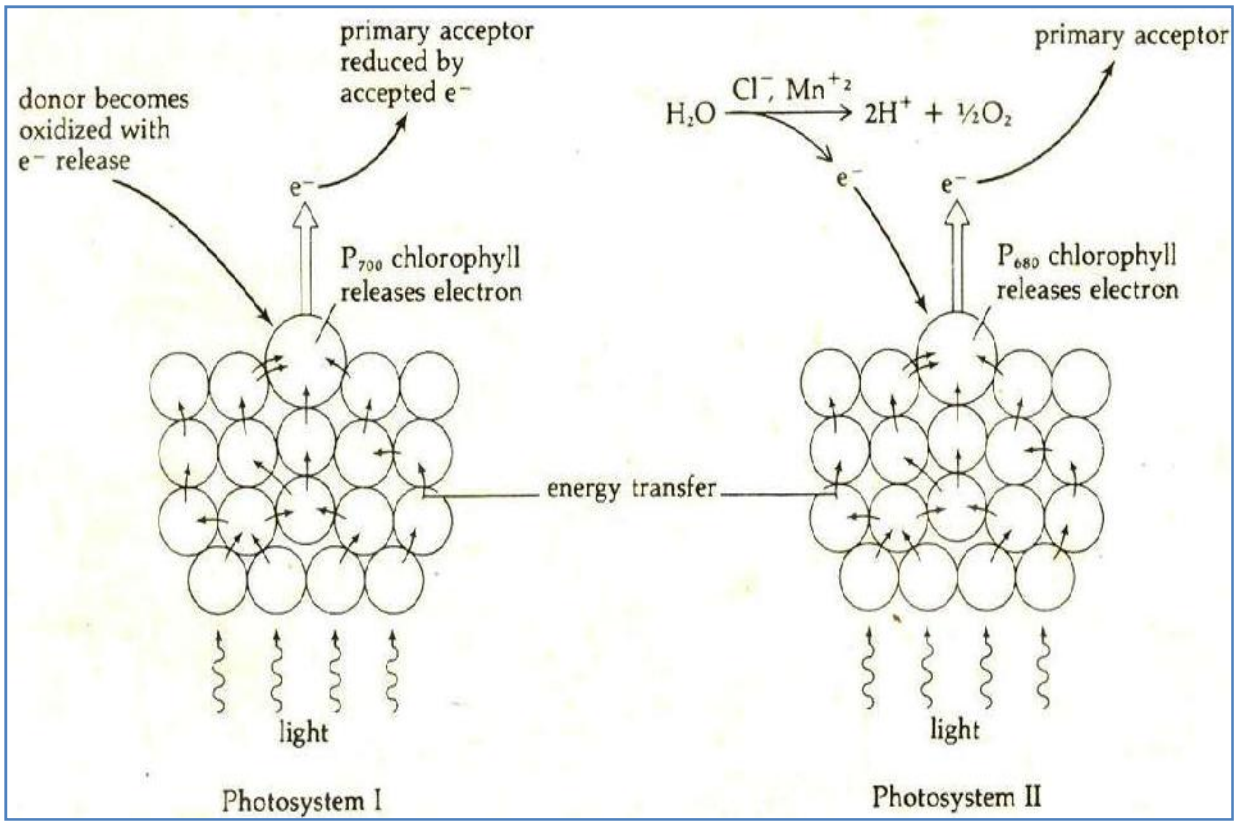
4 light quanta are required to fix one molecule of $CO_2$ and release of one mole of $O_2$. Every $CO_2$ molecules requires $2H_2O$ molecules for reduction i.e. 4 hydrogen atoms. Two light quanta are required for transfer of every atom of hydrogen during photosynthesis. Quantum yield

may be explained on the basis of number of light quanta required to reduce one molecule of $CO_2$ and release of one molecule of $O_2$.

**Quantum yield**

According to Emerson Lewis (1943) 8 quanta of light are required for reduction of one molecule of $CO_2$ and release of one molecule of $O_2$. Therefore, the quantum yield is 1/8 or 12%. Two light quanta are required to excite one electron. It may also be explained as moles of $CO_2$ fixed or $O_2$ evolved in photosynthesis, or electrons transported in the photosynthetic membrane, per mole of quanta absorbed, in the context of gas exchange often restricted to the linear, light-limited part of the photosynthesis–irradiance curve, when measuring chlorophyll fluorescence, it refers to the full range of photosynthetic irradiance. It has been observed that the quantum yield is dropped near the far red region of spectrum. Dropping of quantum yield near far red region of the spectrum which begins at wavelengths greater than 680 nm in green plants and 650 nm in red algae is called **Red drop phenomenon.** This rate of drop in photosynthesis can be rectified by providing light of shorter wave length. This Enhancement of photosynthetic rate under influence of two wavelengths (long and short) is called **Emerson effect. Two pigment systems**

**Emerson effect. Two pigment systems :** In the late 1950s and 1960s, the Emerson effect received a great deal of attention. It became apparent that photosynthesis requires two functioning pigments termed photosystems. Photosystem I is rich in chlorophyll a and contains




carotenoids and less chlorophyll b then does photosystem II. In both the photosystems most of the pigments operate to harvest light energy and transfer it, possibly by resonance, to chlorophyll a molecules located at photochemically active reactive centre termed traps. The active centre pigment for photosystem I consists of chlorophyll a, which absorbs at 703 nm and is called P700. The chlorophyll a collecting pigment at the reactive centre of photosystem II to exhibits an absorption peak at 682 nm and is termed P680. The chlorophyll a molecules (donor molecules) reduces specific electron acceptors (A) and become oxidized themselves. The electron carriers that are thus reduced initiate electron flow and the conversion of light energy to chemical energy (transduction).

**Light harvesting complexes**

Besides PS I and PS II two other green bands are also present. Each band contains chlo.a + chlo.b + little amount of β carotene. All these pigments are protein bound. One band functions with PS I and another with PS II. Function of these bands is to absorb light energy and transfer it to the appropriate pigment system.

**Coordination between PS I and PS II**

According to Jung (1982) each granum has about 200 units of PS I and PS II which functions jointly to transfer electrons from water to NADP. Since they are located a quite apart, certain intermediates are required to carry electrons from PS II to PS I. They are of two types (a) A copper containing proteins called plastocyanin bound loosely to the inside of thylakoid membranes (b) A group of quinones called plastoquinones (PQ). Besides, certain other carriers are also involved.

**Photophosphorylation**

- The addition of phosphate group to ADP under influence of light energy to form ATP.
- The formation of ATP from ADP and inorganic phosphate (Pi) using light energy stored in the proton gradient across the thylakoid membrane**.**

Arnon and others (1954) demonstrated that isolated chloroplasts produce ATP in the presence of light. It was termed as photosynthetic phosphorylation or photophosphorylation. It was shown that the mitochondria are not the alone cytoplasmic organelles involved in the ATP formation. The formation of most ATP in mitochondria takes place by means of process known as oxidative phosphorylation.

Also, ATP formation in chloroplasts differs from that it is independent of respiratory oxidants. In chloroplast light energy is used in the formation of ATP i.e., light energy is converted to chemical energy. ATP is one of the only requirements for carbohydrate production. A reductant must be formed in photosynthesis that will provide the hydrogens or electrons. As

back as 1951, Arnon demonstrated that isolated chloroplasts are capable of reducing pyridine nucleotides when these chloroplasts are exposed to light .The photochemical reaction has to be coupled with an enzyme system capable of utilizing the reduced pyridine nucleotide as quickly as it is formed. It was reported that NADPH is the reduced pyridine nucleotide active in photosynthesis. In the presence of $H_2O$, ADP and orthophosphate (Pi) substrate amounts of NADP were reduced accompanied by the evolution of oxygen as follows:

$$2ADP + 2Pi + 2NADP + 4\ H_20$$

light energy chloroplasts ↓

$$2ATP + O_2 + 2NADPH + 2H_2O$$

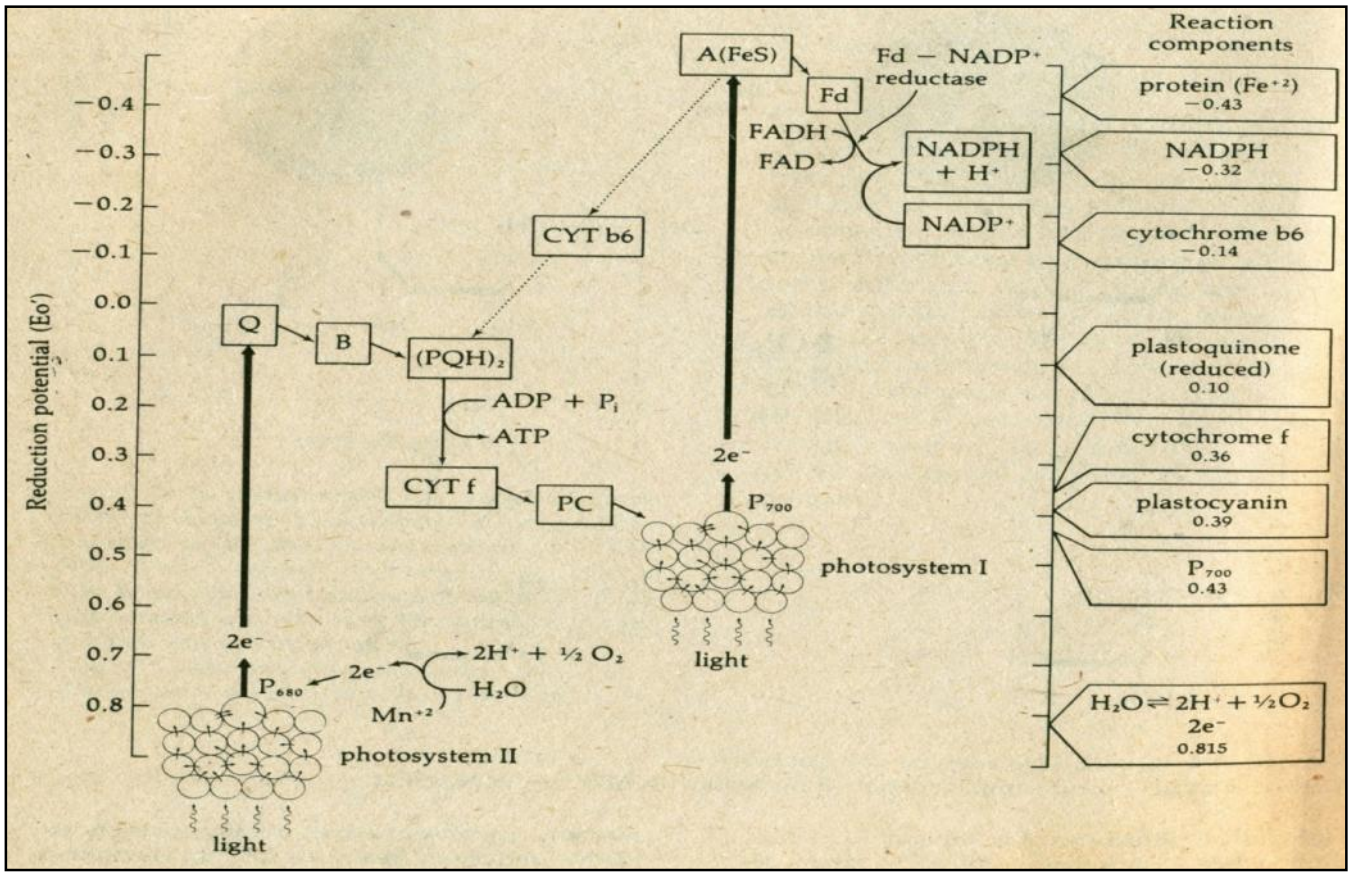
It shows that evolution of one mole of $O_2$ is accompanied by the reduction of the 2 moles of orthophosphate. Together ATP and NADPH provide the energy and reducing power for $CO_2$ fixation and reduction. In bacterial photosynthesis NADP is utilized instead of NADPH.

**Z–Scheme: Electron Transport and Photophosphorylation (Noncyclic Photophosphorylation)**

The Z– scheme illustrates electron transport and the production of NADPH and ATP in chloroplasts. It is called Z scheme due to zig zag pattern of electron flow. The primary flow of electrons within a given granum thylakoid may be initiated almost simultaneously for each photosystem through integrated (coupled) reactions and photolysis of water, which provides the necessary electron flow to produce ATP and NADPH. This integration of two photosystems is most commonly referred to as noncyclic photophosphorylation to describe one means of ATP production in chloroplasts. It is also termed noncyclic electron transport to refer to the manner of electron flow during the process.

In the process after excitation of $P_{700}$ the trap chlorophyll of photosystem I, the electrons are passed on to an unknown primary electron acceptor, believed to be an iron-sulphur protein and designated A (FeS). The electrons are then passed to ferredoxin and ultimately to $NADP^+$, with the formation of $NADPH^+$. Normally the reduced form of NADP is written as NADPH. In fact it should be written like NADPH+ $H^+$. The transfer of electrons to $NADP^+$ creates an electron debit referred to as a hole in photosystem I. However, this deficit is made up by the excitation of $P_{680}$ of photosystem II, subsequently photoejection of electrons and their transport through a system of carriers QB, plastoquinone (PQ), cytochrome f (CYT f), and plastocyanin

(PC). At this point Q and B are unidentified compounds. Figure illustrates that plastoquinone shuttles protons and passes electrons to cytochrome f. At this point ATP is produced. The hole created in photosystem II is filled by electrons that are derived from the splitting (photolysis) of water .Thus the passage of electrons is not in a cyclic manner.

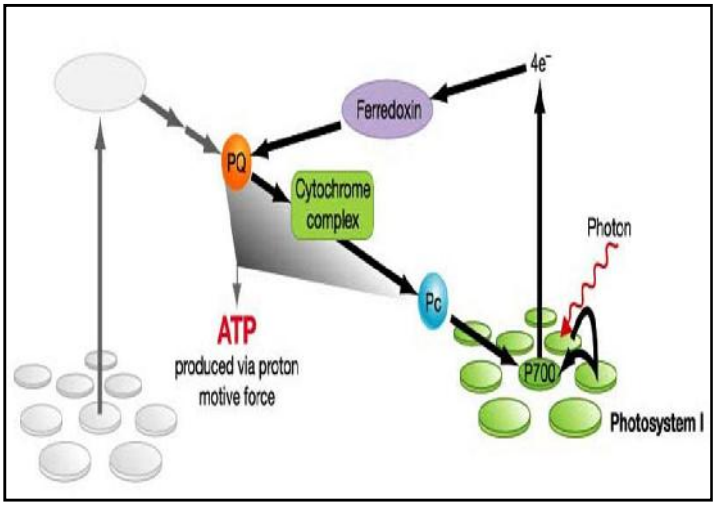



**Cyclic photophosphorylation**

One way of excluding noncyclic photophosphorylation is to illuminate chloroplasts with wavelengths of light greater than 680 nm. Under these conditions only photosystem I is activated and electrons are not removed from $H_2O$, as illustrated by the lack of oxygen evolution under these circumstances. When the flow of electrons from $H_2O$ is stopped, noncyclic photophosphorylation is also stopped and as a consequence $CO_2$ assimilation is retarded as a result of which oxidized NADP is no longer available as an electron acceptor. Activation of photosystem I by wavelengths of light greater than 680 nm causes electrons to flow from $P_{700}$ to A (FeS). When electrons are not passed to $NADP^+$, they may be lost to cytochrome $b_6$ which in turn passes electrons back to $P_{700}$ via cytochrome f and plastocyanin. There is some evidence that

plastoquinone instead of cytochrome $b_6$ may act as the primary acceptor of electrons from A (FeS). This possibility is quite likely because plastoquinone is necessary for proton transport across the thylakoid membrane for the generation of ATP.

There is possibility of production of two ATPs, one between (FeS) and cytochrome $b_6$ and one between cytochrome $b_6$ and cytochrome f which is not very likely without plastoquinone mediation. Nevertheless, the term photophosphorylation is used to denote the cycling of electrons from the donor ( excited $P_{700}$ system ) to an acceptor ( possibly FeS) and back to the $P_{700}$ trap with some generation of ATP. If cyclic photophosphorylation does not indeed operate appreciably in certain organisms, it only produces limited ATP.




**Primary electron acceptors and donors**

In the late 1950s scientists thought that the reduction of $NADP^+$ was associated with a soluble protein factor found in chloroplasts. Arnon and his colleagues (1957) observed that this protein preferentially reduced with the evolution of stoichiometric amounts of oxygen. They called it the NADP - reducing factor which was purified and named photosynthetic pyridine nucleotide reductase (PPNR), since its catalytic activity was only apparent when chloroplasts were illuminated. Tagawa and Arnon (1962) recognized that PPNR is one of a family of nonheme, nonflavin, iron - containing proteins that is universally present in chloroplasts. Generally generic term **ferridoxin** is used to describe these proteins. Scientists have isolated proteins of the ferridoxin family from the chloroplasts of a variety of plants and have assigned them various functions. What we now call methaemoglobin-reducing factor, photosynthetic pyridine nucleotide reductase (PPNR), heme-reducing factor and red enzyme.

Before the discovery of ferridoxin, $NADP^+$ was thought to be the initial electron acceptor of the photosynthetic light reaction. However, neither $NADP^+$ nor ferridoxin is believed to be the

primary acceptor of electrons from $P_{700}$. There is evidence that suggests the existence of an intermediate of an iron-sulphur protein acceptor, A (FeS) between ferridoxin and photosystem I.

In earlier Z- schemes, plastoquinone was designated as the primary electron acceptor from $P_{680}$. In the figure Q stands for the unknown primary acceptor that quenches the fluorescence of chlorophyll a. Plastoquinone is reduced by transfer of electrons from Q through B, the latter being a secondary unidentified acceptor that is associated with a photosystem II membrane protein.

The reduced plastoquinone is oxidized by the transfer of an electron to cytochrome f. Either cytochrome f or plastocyanin (Cu containing protein) is the immediate electron donor to photooxidized $P_{700}$. Both compounds are found associated with the photosynthetic tissues of algae and higher plants and both compounds have redox potentials close to that of $P_{700}$ (about 0.43). However, there is some indication that plastocyanin is located closer than cytochrome f to the photoreaction centre $P_{700}$ of photosystem I. Therefore, plastocyanin is considered the immediate electron donor to phtooxidized $P_{700}$. Cytochrome f, in this case, would transfer electrons to plastocyanin.

**Proposed mechanisms of ATP formation**

Electron flow and the photophosphorylation of ADP to ATP and $H_2O$ are distinct processes that are coupled, or have energy transferred from one to the other, by some common reactant. Evidence for this coupling is based on several observations. 1. In the presence of coupling agents, ATP formation can be inhibited but electron transport continues and often shows an increase in rate. When the coupler is removed, ATP formation resumes in pace with electron transport. 2. When electron transport is impeded or blocked by certain herbicides, such as the triazins, triazinones, biscarbamates and (3-[3,4 – dichlorophenyl [-1,1-dimethylurea), phosphorylation is also inhibited. 3. Scientists have commonly observed simultaneous oxidation of NADPH (NADH in respiration) and FADH in ATP formation.

Scientists have proposed three mechanisms of ATP production.

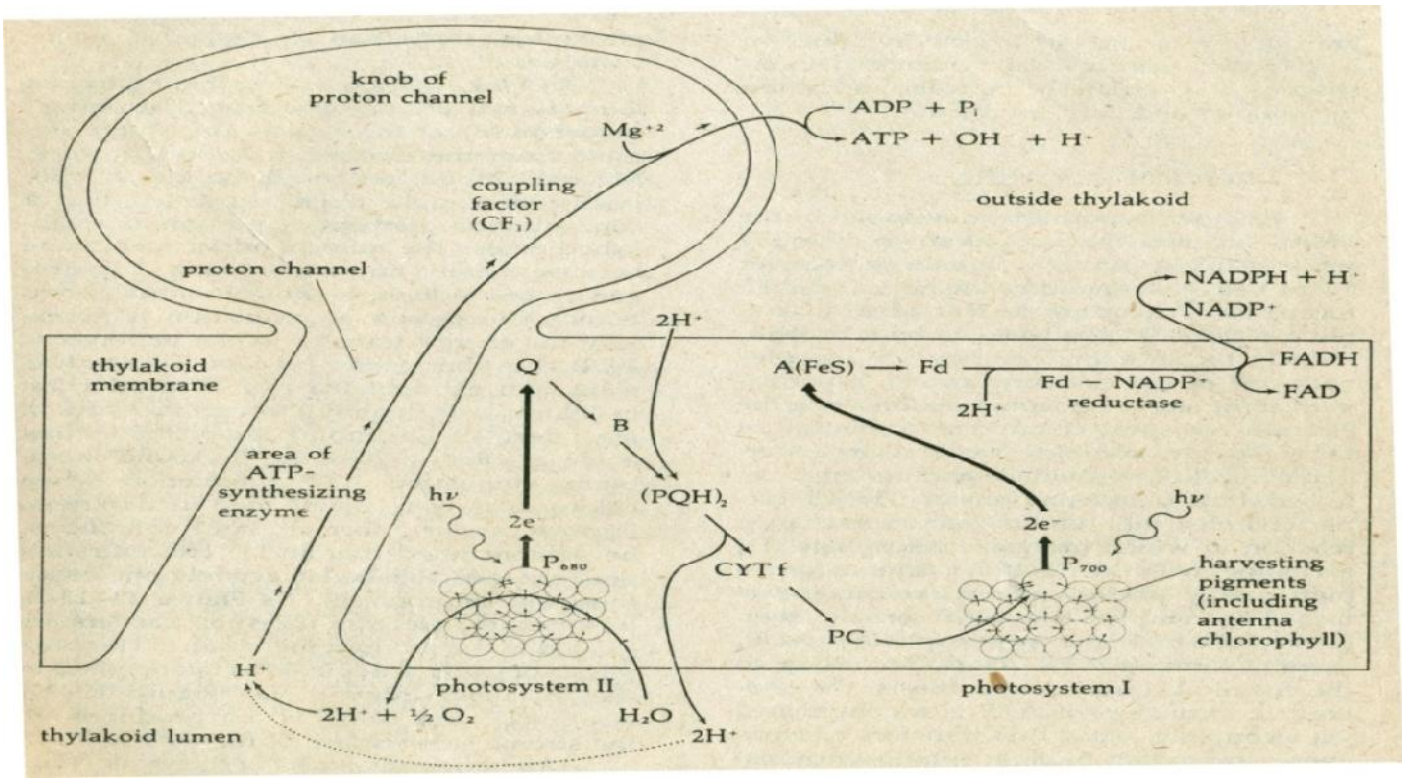



**Fig- Granum thylakoid membrane illustrating location of photophosphorylation and coupling of electron flow to ATP production**

**Conformational coupling**

It is promised by the idea that the membranes of the mitochondria or chloroplast thylakoids undergo structural changes and that these changes presumably induce high energy states, or conformations, that favour the release of energy for the ATPase catalyzed production of ATP. Although ATPase normally catalyses ATP decomposition to ADP and inorganic phosphate (Pi). It will work in the reverse direction when sufficient energy is available. Electron micrographs illustrate differences in structure of membranes (mostly mitochondrial membranes) during organelle activity.

**Chemical coupling**

Another hypothesis, developed in the 1960s suggests that an unknown coupling protein might act as an energy transfer agent between electron transport and ATP formation. According to this idea, a coupling factor (CF) believed to be a protein, initially forms a high energy CF complex with one of the electron carriers, a participant at the site of phosphorylation along the

electron transport chain. The formation of CF- carrier- complex involves an endergonic reaction provided with energy released during electron transfer. The CF-carrier -complex then enters into an exchange reaction in which inorganic phosphate (Pi) exchanges with electron carrier to form a high energy phosphorylated coupling factor (CF-P) complex which releases the high energy phosphate to ADP, thereby forming the ATP. Thus, according to the chemical coupling hypothesis, the endergonic formation of ATP is accomplished via a coupling factor that transfers electron energy promoted by light (photosynthesis) or oxidation of organic chemicals (respiration).

**Chemiosmotic coupling**

This hypothesis is the most widely accepted explanation for oxidative phosphorylation in mitochondria and has recently gained importance as an explanation for phosphorylation in thylakoid membranes. In 1961, after observing that hydrogen ions are actively released from respiring mitochondria at the expense of energy received from the electron transport process. Mitchell (1961, 1978) proposed the idea of chemiosmotic coupling. He suggested that a concentration gradient of protons is established across the mitochondrial membrane because there is an accumulation of hydrogen on one side of the mitochondrial membrane. The proton accumulation is necessary for energy transfer to the endergonic ADP phosphorylation process. Jagendorf (1975) demonstrated that a $_{P}H$ gradient across the thylakoid membrane stimulated ATP production when chloroplasts were maintained in darkness. He also demonstrated that under normal light conditions an $H^{+}$ concentration gradient is established in actively photosynthesizing chloroplasts. As figure illustrates, the electron carriers are located in the granum membrane. ATP and NADPH are produced on the stroma side surface of the thylakoid. An important aspect of the model is the mobility of plastoquinone. This carrier presumably transfers electrons to cytochrome f and in addition pick up $H^{+}$ ions on the outside and releases protons to the thylakoid channel. The transfer of protons to the inside and the production of protons from the photolysis of water incurs buildup of protons inside and a $_{P}^{H}$ gradient across the thylakoid membrane to the outside (stroma side), where the hydrogen concentration is relatively low. The membrane itself is not permeable to protons concentrated on the channel side, which represents a source of energy. It is believed that protons flow from the inside of the stroma side

of the membrane through special pathways of CF (stalks) that terminate as knobs at the outer (stroma side) surface. These stalks and knobs are the sites of photophosphorylation. The proton flow along the gradient provides the necessary energy for the following reaction:

$$ADP + Pi \xrightarrow[ATPase]{} ATP + H_2O + 8{,}000 \text{ cal/ mole}$$

The proton flow and phosphorylation are thought to be brought together (coupled) by the activity of the enzyme ATPase (also called coupling factor) which is associated with the destruction of ATP, but it will operate in the reverse situation as long as sufficient energy is supplied (in this case from the proton flow). For every two electrons passing through the transport system, two protons are transported by the reduced plastoquinone, a water molecule is photolyzed. Theoretically, one molecule of ATP is produced for every three protons passing through the CF.

The light reaction phase of photosynthesis may be summarized by the following equation, which represents the photochemical, photophosphorylation, photoreduction and photooxidation (splitting of water) events:

$$2H_2O + 2NADP^+ + (ADP)n + (Pi)\,n \xrightarrow{\text{chloroplasts}} (ATP)n + 2NADPH + 2H^+ + O_2$$

The summary equation also indicates that the stoichiometry of the overall equation is not exact, particularly for ATP production and the quanta required .We do not know the number of ATP molecules produced per oxygen molecule liberated. Some investigators claim 2 molecules of ATP are produced for every oxygen molecule liberated, and others maintain 4. According to plant scientists 8 to 12 quanta (photons) appear to be necessary to produce NADPH and ATP sufficient for $CO_2$ fixation. Approximately 2NADPH and 3ATP molecules are required to incorporate 1 molecule of $CO_2$ into sugar phosphate.

**Difference between cyclic and non-cyclic photophosphorylation**

| **S. No** | **Cyclic** | **Non-cyclic** |
|---|---|---|
| 1. | Only PS I participates | PS I and PS II participate. |
| 2. | PS I gets back electron in a cyclic fashion. | PS I gets back electron from PS II and PS II from water. |

| | | |
|---|---|---|
| 3. | Water does not participate hence No $O_2$ evolution. | $O_2$ evolved as water participates. |
| 4. | Found in bacteria. | Found in green plants. |

**Summary of light reaction**

- Eight light photons are required to oxidize $4H_2O$.
- $2\ e^-$ and $1\ H^+$ are required to reduce 1 molecule of NADP to $NADPH_2$.
  $1H^+$ remains in the medium.
- Transfer of 1 electron from $H_2O$ to NADP requires 2 photons as excitation of both the photosystems is necessary.
- Photolysis of $4H_2O$ produces $2H_2O$, $O_2$, $4\ e^-$ and $4H^+$.
- Reduction of 1molecule of $CO_2$ requires $2NADPH_2$ and 3ATP.
- 4 molecules of water after photolysis forms 3ATP and $2NADPH_2$ and cycle repeats 6 times producing 18 ATP and 12 $NADPH_2$ during light reaction of photosynthesis.

## 14. LECTURE NOTES

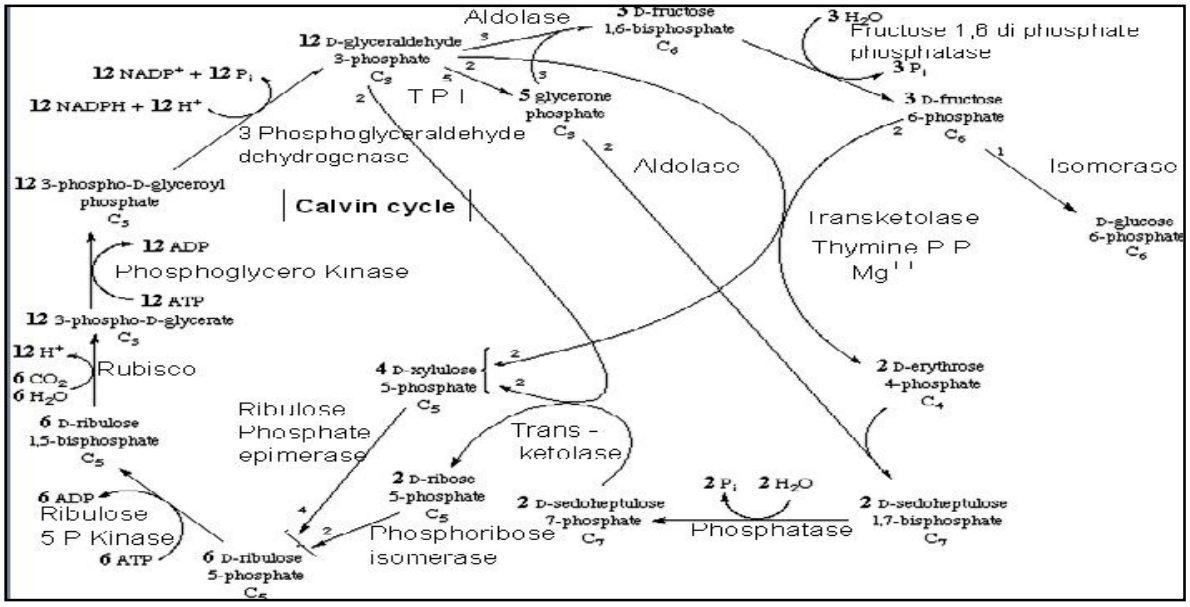
### Calvin cycle, Hatch and Slack pathway.

**Dark reaction**

$CO_2$ is fixed in one of the following pathways in green plants. 1. $C_3$ pathway or Calvin cycle 2. $C_4$ pathway or Hatch and Slack cycle. 3. $C_2$ or Glycolate pathway. 4. Crassulacean acid metabolism (CAM plants) .

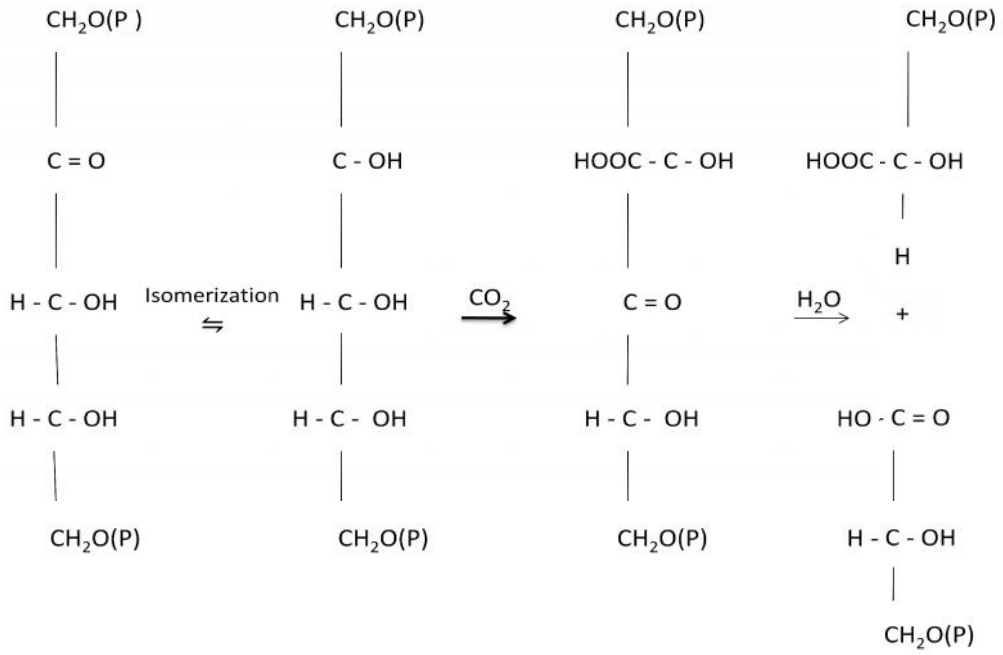
**$C_3$ pathway or Calvin cycle**

- First stable compound formed after the entry of $CO_2$ in plant system is triose sugar viz; Phosphoglyceric acid (PGA) (Calvin 1961).
- Pathway of photosynthetic $CO_2$ assimilation beginning with carboxylation of RuBP by Rubisco.
- The biochemical pathway for the reduction of $CO_2$ to carbohydrate. The cycle involves three phases, the carboxylation of ribulose-1,5-bisphosphate with atmospheric $CO_2$ catalyzed by rubisco, the reduction of the formed 3-phosphoglycerate to triose phosphate by 3-phosphoglycerate kinase and NADP-glyceraldehyde -3-phosphate dehydrogenase, and the regeneration of ribulose-1,5-bisphospate through the concerted action of ten enzymatic reactions.



It is called $C_3$ pathway because the first stable compound formed after the entry of $CO_2$ in plant system is triose sugar i.e. PGA. Calvin by using isotope of carbon $C^{14}$ could identify various products formed during reduction of $CO_2$ by paper chromatography and radio autography. Calvin (1961) later on got noble prize for this work. Chromatography is a technique for separation of compounds present in a small mixture. On the other hand radioautography is a technique to find out isotopes in a particular source. Different radioactive isotopes emit different types of radiation. $C^{14}$ emits β rays. Calvin worked on these aspects in blue green algae chlorella.

Six molecules of $CO_2$ react with 6 molecules of Ribulose 1, 5 biphosphate or bis phosphate to form 12 molecules of 3PGA. The reaction is catalysed by RuBP carboxylase . First $CO_2$ is added to a 5C sugar to form 6C sugar which then splits into two molecules of 3 carbon compound.




RuBP Enediol of RuBP β Keto acid intermediate 3PGA

The cleavage of β Keto acid intermediate takes place by water between $C_2$ and $C_3$ of ribulose chain.

In next step 12 molecules of 3PGA are phosphorylated to 12 molecules of 1-3 di phospho glyceric acid. The reaction is catalyzed by phospho glycero kinase

$$12\ 3PGA + 12\ ATP \xrightarrow{\text{Phosphoglycerokinase}} 12\ 1,3\ \text{di phospho glyceric acid} + 12\ ADP$$

In the next step 12 molecules of 1, 3 di phospho glyceric acid reduced to 12 molecules of 3 phospho glyceraldehyde by 12 molecules of NADPH produced in the light phase of photosynthesis.

$$12\ 1,3\ \text{diphospho glyceric acid} + 12\ NADPH_2 \xrightarrow{\text{3 Phospho glyceraldehyde dehydrogenase}} 12\ 3\ P\ \text{glyceraldehyde} + 12\ NADP$$

Five molecules of 3 phospho glyceraldehyde are isomerized to five molecules of dihydroxy acetone phosphate (5 molecules) in presence of triose phosphate isomerase.

$$5\ 3\ \text{phosphoglyceraldehyde} \xrightarrow{\text{Triose phosphate isomerase}} 5\ \text{Dihydroxy acetone phosphate}$$

$$6RuBP + 6CO_2\ (5C) \xrightarrow{6H_2O} \beta\ \text{Keto acid}\ (6C) \longrightarrow 12\ \text{Mols. 3PGA}\ (3C)$$

The 3 molecules of 3 phosphoglyceraldehyde condensed with 3 dihydroxy acetone phosphate to form 3 molecules of fructose 1, 6 diphosphate in presence of aldolase which are then dephosphorylated to 3 mol. of fructose 6 P in presence of phosphatase.

$$3\ \text{Mols 3 phosphoglyceraldehyde} + 3\ \text{Dihydroxy acetone phosphate} \xrightarrow{\text{Adolase}} 3\ \text{Fructose 1 - 6 diphosphate} \xrightarrow[3H_2O]{\text{Phosphatase}} \text{Fructose 6 P} + 3\ H_3PO_4$$

Two molecules of 3 phosphoglyceraldehyde reacts with 2 mol. of fructose 6 phosphate to form 2 mol. of xylulose 5 P and 2 mol. of erythrose 4 P in presence of transketolase and thymine pyrophosphate

$$2\text{-}3\ \text{Phosphoglyceraldehyde} + 2\ \text{Fructose 6} \xrightarrow{\text{Transketolase}} 2\ \text{Xylulose 5 P} + 2\ \text{Erythrose 4 P}$$

phosphate

Two mol. of erythrose 4 P reacts with two mol. of 3 phosphoglyceraldehyde to form two sedoheptulose 1-7 diphosphate in presence of aldolase.

$$2 \text{ Erythrose 4 P} + 2 \text{ Dihydroxy acetone P} \xrightarrow{\text{Aldolase}} 2 \text{ Sedoheptulose 1-7 diphosphate}$$

Two molecules of sedoheptulose 1-7 diphosphate then dephosphorylated to two molecules of sedoheptulose 7 P in presence of phosphatase.

$$2 \text{ Sedoheptulose 1-7 di P} + 2H_2O \xrightarrow{\text{Phosphatase}} 2 \text{ mol. Sedoheptulose 7 P} + 2H_3PO_4$$

Two mol. of sedoheptulose 7 P condensed with the remaining two mol. of 3 P Glyceraldehyde in presence of enzyme transketolase to produce two mol. of Xylulose 5 P and two molecules of ribose 5 P.

$$2 \text{ mol Sedoheptulose 7 P} + 2 \text{ 3 phosphoglyceraldehyde} \xrightarrow{\text{Transketolase}} 2 \text{ Xylulose 5 P} + 2 \text{ Ribose 5 P}$$

All 4 molecules of Xylulose 5 P obtained from different reactions undergo epimerization in presence of ribulose 3 epimerase to form 4 mol. of ribulose 5 P.

$$4 \text{ Xylulose 5 P} \xrightarrow{\text{Ribulose phosphate 3 epimerase}} 4 \text{ Ribulose 5 P}$$

Two mol. of ribose 5 P undergo isomerization in presence of phosphoribose isomerase to form 2 mol. of ribulose 5 P.

$$2 \text{ Ribose 5 P} \xrightarrow{\text{Phosphoribose isomerase}} 2 \text{ Ribulose 5 P}$$

Six molecules of Ribulose 5 P are formed up to this stage. In the final step all 6 molecules of ribulose 5 phosphate are phosphorylated at the expense of 6 mol. of ATP in presence of enzyme phosphoribulo kinase to form 6 mol. of carbon acceptor ribulose 1-5 diphosphate. Then all 6 the molecules of ribulose 1-5 diphosphate are regenerated reenter into cycle.

Whole reaction can be written as:

$$6CO_2 + 12 \text{ NADPH} + 12 H^+ + 18 \text{ ATP} + 11 H_2O \longrightarrow 1 \text{ Mol. F} - 6 \text{ P} + 12 \text{ NADP}^+ + 18 \text{ ADP} + 17$$

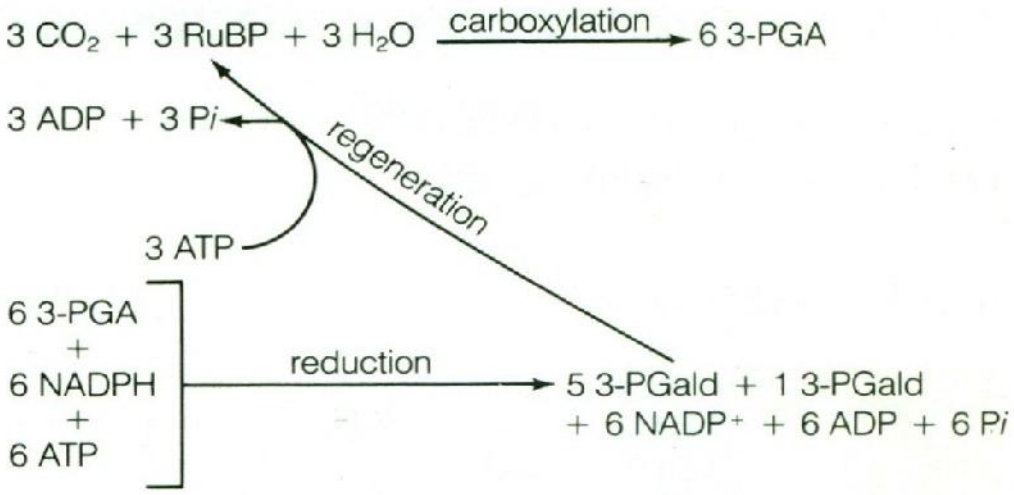
Pi

**Synthesis of carbohydrate**

Out of 3 mol. of fructose 6 phosphate one molecule is isomerised under the influence of enzyme isomerase to form glucose 6 phosphate. Dephosphorylation of glucose or fructose 6 P in the influence of phosphatase enzyme forms glucose or fructose which further combines to form sugar or starch.

**Calvin cycle occurs in three main parts:**

1. **Carboxylation** - Which involves addition of $CO_2$ and $H_2O$ to form two molecules of 3 PGA.
2. **Reduction** - In which COOH group in 3 PGA is reduced to an aldehyde group (3 P Galdehyde) .
3. **Regeneration of RuBP –** Out of the entry for every $6CO_2$ molecules the output is one mole of glucose and rest go to the regeneration of RuBP.

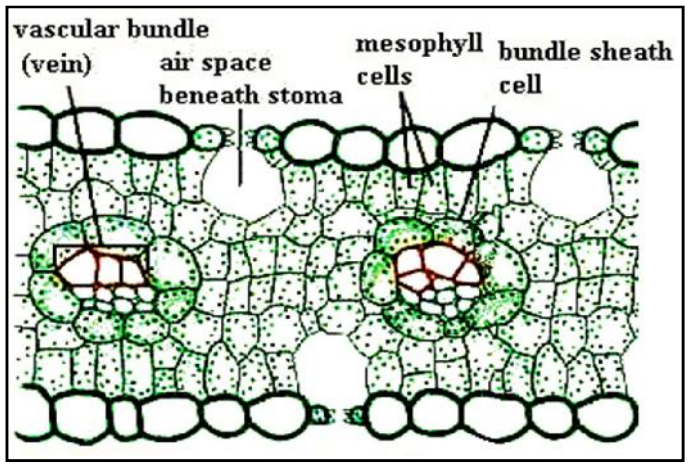



**Hatch and Slack pathway ($C_4$ cycle)**

summary:

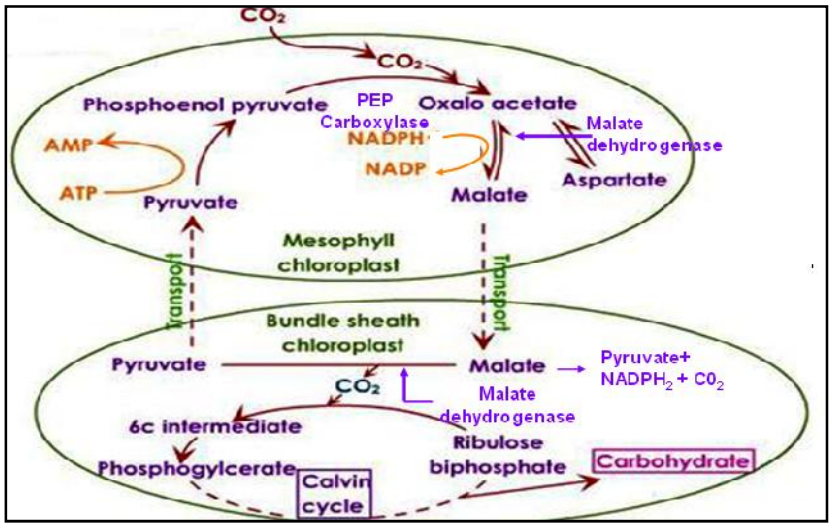
$$3\,CO_2 + 3\,H_2O + 3\,RuBP + 9\,ATP + 6\,NADPH \longrightarrow 1\,3\text{-PGald} + 6\,NADP^+ + 9\,ADP + 9\,Pi$$

- The photosynthetic carbon metabolism of certain plants in which the initial fixation of $CO_2$ and its subsequent reduction takes place in different cells, the mesophyll and bundle sheath cells, respectively. The initial carboxylation is catalyzed by phosphoenolpyruvate carboxylase, (not by rubisco as in $C_3$ plants), producing a four-carbon compound (oxaloacetate), which is immediately converted to malate or aspartate.




Hatch and Slack (1966) found that the first product of this cycle is 4 carbon acid. This cycle occurs mainly in sugarcane, maize, grasses, atriplex etc. $C_4$ plants possess Kranz anatomy. In this case the mesophyll cells are not differentiated into palisade and spongy parenchyma. Vascular bundles are surrounded by layers of radially arranged parenchymatous cells. The sheath appears like a wreath, hence called Kranz (wreath) anatomy .Phospho enol pyruvate is the initial acceptor of $CO_2$.

$$PEP + CO_2 + H_2O \xrightarrow{\text{PEP Carboxylase}} \text{Oxalo acetic acid}$$




Initially $CO_2$ is accepted by Phospho enol pyruvate (PEP) under influence of PEP carboxylase enzyme forming aspartate. Malate is produced by activity of malic dehydrogenase in presence of $NADPH_2$. Malate is transferred to chloroplast of bundle sheath. Here malate is decarboxylated under influence of malate dehydrogenase to produce pyruvate, $CO_2$ and $NADPH_2$. $NADPH + H^+$ travels back to mesophyll cells to regenerate malate, while pyruvate also travels back to mesophyll cells, where it utilizes the light generated ATP to produce PEP

again. $CO_2$ released by decarboxylation of malate is fixed in the bundle sheath cells by $C_3$ pathway (accepted by RuBP).

In $C_4$ plants there are two carboxylations, one by atmospheric $CO_2$ which forms dicarboxylic acids and second by internally generated $CO_2$ entering the RuBP. In this case $C_3$ and $C_4$ both pathways operate. $C_3$ and $C_4$ pathways are delimited to bundle sheath cells and mesophyll cells, respectively. Because there is carboxylations at two sites, the pathway is also known as dicarboxylation pathway.

Chollet and Orgen (1975) found three categories of $C_4$ plants.

1. Fixation of $CO_2$ by PEP which forms oxalo acetate then malate Ex. maize, sugarcane.
2. Oxalo acetate gets converted into aspartate in mesophyll cells and it is transported to bundle sheath. In bundle sheath cells aspartate is reconverted to oxalo acetate which is then converted to pyruvate and $CO_2$ Ex. *Panicum maximum*, *Chloris guyana.*
3. Aspartate produced in mesophyll cells is transported to bundle sheath cells where it gets transaminated to oxalo acetate first and then gets reduced to malate in mitochondria using NADH. The malate is decarboxylated to produce pyruvate and $CO_2$ Ex. *Atriplex spongiosa*.

**Significance of $C_4$ pathway**

1. They possess higher rates of photosynthesis due to higher affinity of PEP carboxylase to $CO_2$.
2. They can carry on photosynthesis even under low $CO_2$ concentrations (10ppm).
3. Even under almost closed conditions of stomata $C_4$ plants can continue to photosynthesize.
4. There is almost negligible photorespiration.

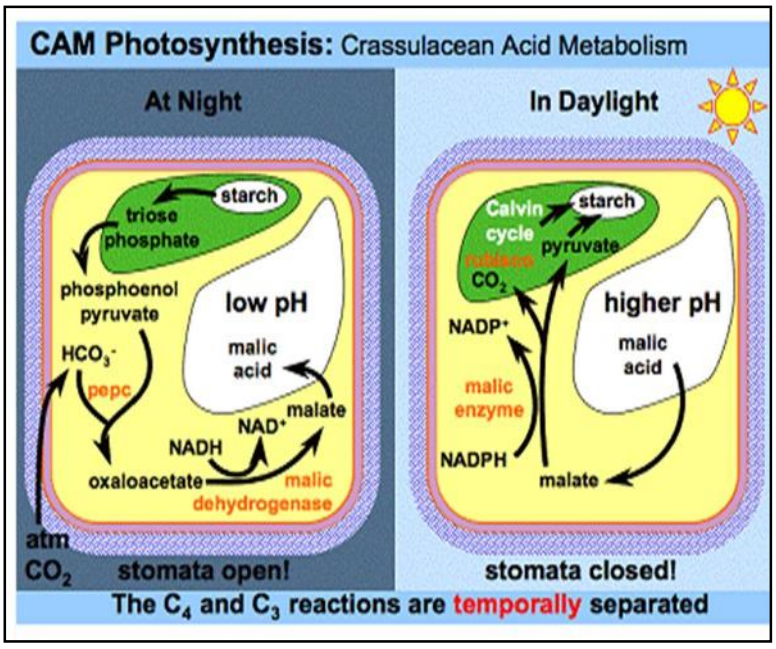
It is not necessary that $C_4$ pathway is always more efficient than $C_3$ pathway but most of the time $C_4$ pathway leads to better utilization of available $CO_2$ in $C_3$ fixation . $C_4$ pathway itself does not produce carbohydrates. It is only contributory pathway for $C_4$ cycle.

## 15. LECTURE NOTES

## CAM pathway,Bacterial photosynthesis, photorespiration, factors affecting rate of photosynthesis

**Crassulacean Acid Metabolism (CAM Pathway )**

- Stomatal opening in night due to accumulation of malic acid which acts as strong solute and closing during day as a result of disappearance of malic acid from stomatal guard cells.
- Plants that fix $CO_2$ during the night into a four-carbon compound (malate) that, after storage in the vacuole, is transported out of the vacuole and decarboxylated during the day. The $CO_2$ released is assimilated in the Calvin cycle in the chloroplast stroma.




Many plants belonging to families like Crassulaceae, Orchidaceae, Bromeliaceae, Liliaceae, Asclepiadaceae, Vitaceae etc. Examples Agave, Kalanchoe, Sedum found in arid regions. In such plants stomata open during night and close during day which reduces the rate of transpiration thereby helps in water conservation.

In night $CO_2$ is fixed by phospho enol pyruvic acid under the influence of PEP carboxylase enzyme. This leads to the formation of oxalo acetic acid which is then reduced to $NADPH_2$ under influence of malic dehydrogenase enzyme. Due to accumulation of malic acid in guard cells the osmotic concentration of guard cells of stomata becomes higher resulting in withdrawal of water from the surrounding cells increasing the turgor pressure of guard cells which results in opening of stomata, whereas, in day malic acid gets converted to $CO_2$ and pyruvic acid. $CO_2$ is accepted by RuBP in Calvin cycle and pyruvic acid is utilized in the formation of carbohydrates. Due to disappearance of malic acid in

day stomata remain closed. Due to formation of malic acid in dark the $_{P}H$ should become low and stomata should remain closed but malic acid being a strong solute increases the osmotic pressure of guard cells. In such plants starch sugar mechanisms do not operate.

**Bacterial photosynthesis**

Certain bacteria also manufacture food material through light energy. Bacterial pigments are contained in small structures called chromatophores. Such bacteria are generally anaerobic bacteria and use $H_2S$ or some other compound for electron source instead of water. In their photosynthesis no $O_2$ evolution takes place. Ex- Chlorobacterium, Sulphur bacteria- Chromatium, Nonsulphur bacteria- Rhodospirillum. There are certain colourless bacteria which can manufacture food material from $CO_2$ and water but for this purpose they make use of chemical energy released during biological oxidation of certain inorganic substances. Due to lake of chlorophyll they cannot use light energy. This process of food synthesis is called chemosynthesis. Chemosynthetic bacteria are aerobic. They are nitrifying like Nitrosomonas, Nitrobacter, Iron bacteria- Spirophyllum, Hydrogen bacteria like Bacillum pantotrophus.

**Difference between $C_3$ & $C_4$ plants**

| **S.N.** | **Character** | **$C_3$ Plants** | **$C_4$ Plants** |
|---|---|---|---|
| 1. | $CO_2$ Acceptor | RuBP (Ribulose bi or bis phosphate) | PEP (Phospho -enol pyruvate) |
| 2. | First stable product | PGA(Phospho glyceric acid) | Oxaloacetate |
| 3. | Type of chloroplast | One | Dimorphic, bundle sheath chloroplasts lack grana, mesophyll cells have normal chloroplast |
| 4. | Leaf anatomy | Normal | Kranz (German) |
| 5. | Pigment system | All chloroplasts have PS I and PS II | Bundle sheath chloroplasts lack PS II, hence depends on mesophyll cell chloroplasts for supply of NADH |
| 6. | Enzymes of $C_3$ Pathway | Found in mesophyll cells | Found in bundle sheath cells |

| | | | |
|---|---|---|---|
| 7. | $CO_2$ compensation point | 150- 500ppm | 0-10 ppm |
| 8. | Photorespiration | Present | Almost absent |
| 9. | Net rate of PS in full sunlight | 15-35 mg $CO_2$ /dm $^2$ of leaf | 40-80 mg $CO_2$ /dm $^2$ of leaf |
| 10. | Saturation intensity | 1000-4000 ftc. | Difficult to reach saturation |
| 11. | Bundle sheath cells | Not prominent | Very prominent |
| 12. | $CO_2$ fixation | $C_3$ Pathway | Both $C_3$ and $C_4$ pathways |
| 13. | Higher $O_2$ rate | Inhibits PS | No effect on PS |
| 14. | Temperature optimum | 10-25 $^0$C | 30-45 $^0$C |
| 15. | ATP molecules required to synthesize one molecule of glucose | 18 | 30 |

**Photosynthetic features of $C_3$, $C_4$ and CAM plants**

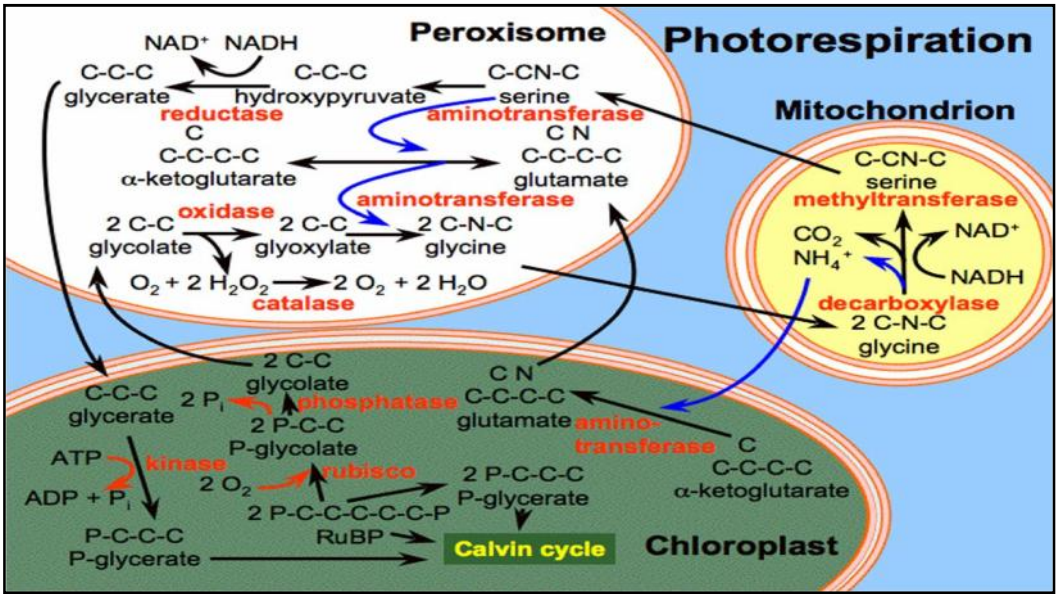
| **Features** | **$C_3$ Plants** | **$C_4$ Plants** | **CAM Plants** |
|---|---|---|---|
| Kranz anatomy | No | Yes | No |
| $CO_2$ acceptor | RuBP | PEP | PEP |
| $CO_2$ fixation product | 3-PGA | Oxaloacetic acid and other $C_4$ acids | Oxalo acetic and other $C_4$ acids |
| Carboxylase | RuBPcarboxylase | PEP carboxylase, RuBP carboxylase | PEP carboxylase, RuBP carboxylase |
| $CO_2$ fixation | Light | Light | Darkness- $C_4$ cycle, light – $C_3$ cycle |
| $O_2$ inhibition of photosynthesis | Yes | No | Yes |
| Chloroplast | One structure | Two structures | ? |
| Photorespiration | High | Low (Bundle sheath cells only) | Very low |
| Transpiration | High | Low | Very low |

| | | | |
|---|---|---|---|
| Productivity | Low or High | High | Low or High |
| $CO_2$ compensation point | High (25-100ppm) | Low (0-10 ppm) | Low (0-5 ppm) |

Although $CO_2$ fixation can take place in darkness, the amount of $CO_2$ fixed is greater in the light because of availability of ATP and NADPH from the light reactions and because the stomata are open and facilitate gas exchange.

**Photorespiration**

- Increase of respiration rate induced by higher light intensities and $O_2$ with reduced $CO_2$ concentration, takes place in cooperative way among chloroplast, peroxysomes, and mitochondria. Glycolate being the substrate. Examples – Paddy, Soybean, Wheat etc.
- Production of $CO_2$ in the metabolic pathway that metabolizes the products of the oxygenation reaction catalyzed by Rubisco.
- Uptake of atmospheric $O_2$ with a concomitant release of $CO_2$ by illuminated leaves. Molecular oxygen serves as substrate for rubisco and the formed 2-phosphoglycolate enters the photorespiratory carbon oxidation cycle. The activity of the cycle recovers some of the carbon found in 2-phosphoglycolate, but some is lost to the atmosphere.




Increase c

optimum temperature for photorespiration is $25^0$- $35^0$ C.

**Mechanism**

In normal respiration the substrate is sugar or glucose, whereas in photorespiration the substrate is 2C compound called glycolic acid. Under normal conditions Rubisco (Ribulose 1, 5 biphosphate) catalyses fixation of $CO_2$ in Calvin cycle. Under high $O_2$ and lower $CO_2$ concentration rubisco oxidises Rubp in one molecule of 3C PGA and one molecule of 2C phosphoglycolic acid which ultimately forms glycolic acid. Therefore, Rubisco is also called RuBP oxinase.

(a) Carboxylase activity RuBP $\xrightarrow[CO_2 + H_2O]{\text{Rubisco}}$ 2 Moles of 3 PGA

(b) Oxinase activity RuBP $\xrightarrow[+ O_2]{\text{Rubisco}}$ Phospho glyceric acid (2C) + 3 PGA (3C)

In the next step phosphoglycolic acid undergoes decarboxylation to form glycolic acid. The reaction is catalyzed by enzyme phosphatase.

$$\text{Phosphoglycolic acid} + H_2O \xrightarrow{\text{Phosphatase}} \text{Glycolic acid} + \text{Phosphoric acid}$$

The glycolic acid which is formed in chloroplast is transported out into the peroxisomes where glycolic acid is converted into glyoxylic acid in presence of enzyme glycolate oxidase.

$$\underset{\text{Glycolic acid}}{CH_3 - \underset{OH}{\underset{|}{C}}OOH} + O_2 \longrightarrow \underset{\text{Glyoxylic acid}}{H\,C - \underset{O}{\underset{||}{C}}OOH} + H_2O_2$$

Here glycolic acid oxidase transfers electrons (present in H atoms) from glycolate to $O_2$ reducing $O_2$ to $H_2O_2$. Then $H_2O_2$ is broken down to $H_2O$ and $O_2$ by catalase. In the next step glyoxylic acid is converted into glycine under the influence of enzyme glutamate glyoxylate amino transferase (transamination reaction).

$$\text{Glyoxylic acid} + \text{Glutamic acid} \longrightarrow \text{Glycine} + \alpha \text{ Keto glutaric acid}$$

Glycine which is formed in peroxysomes is transported to mitochondria via cytoplasm. Glyoxylic acid may also form glycine by serine by transfer of amino group to it from serine instead of glutamic acid.

$$\text{Glyoxylic acid + Serine} \xrightarrow{\text{Serine amino transferase}} \text{Glycine + Hydroxy Pyruvic acid}$$

In the mitochondria two molecules of glycine joins together to form a molecule of serine. $CO_2$ and $NH_3$ are also released in this reaction.

$$2\ \text{Glycine} + H_2O + \text{NAD} \longrightarrow \text{Serine} + CO_2 + NH_3 + \text{NADH}$$

It is the stage of formation of serine that light induced $CO_2$ liberates. Serine is then transported out of mitochondria and into peroxysomes where it is converted into hydroxy pyruvic acid and then to glyceric acid. Finally glyceric acid is transported to chloroplast where it undergoes phosphorylation by ATP to form 3 PGA. The molecules of 3 PGA join the pool in the $CO_2$ reduction cycle. In $C_4$ plants the rate of photorespiration is almost nil because $CO_2$ concentrates in bundle sheath cells. The positive movement of $CO_2$ from atmosphere towards bundle sheath cells is called $CO_2$ pump.

**Difference between respiration and photorespiration**

| S.No | Feature | Normal respiration | Photorespiration |
|---|---|---|---|
| 1. | Respiratory substrate | Carbohydrates, fats or proteins | Glycolate |
| 2. | Biosynthesis of the substrate produced | Stored or recently | Always recently produced |
| 3. | Site of occurrence in cells | Cytoplasm and mitochondria | Chloroplasts,mitochondria and peroxisomes |
| 4. | $H_2O_2$ | Not produced | Produced |
| 5. | ATP | Formed | Not formed |
| 6. | NAD & NADH | NAD reduced to NADH | NADH oxidized to NAD |
| 7. | Transamination | Does not occur | Takes place |
| 8. | $O_2$ concentration | Not dependant totally on $O_2$ concentration | Dependent totally on $0_2$ concentration |
| 9. | Temperature Sensitivity | Not very sensitive | High acceleration of the process between 25-30$^0$C |
| 10. | Occurrence in organisms | All living cells | Only in green plants |
| 11. | Occurrence in time | All times | Only in day time (in presence |

| | | | |
|---|---|---|---|
| | | | of light) |

**Significance of photorespiration**

- It protects the chloroplast against the light destruction. It consumes $O_2$ thereby helps to prevent build up of $O_2$ accumulation in chloroplast which may destroy the chloroplast membrane.
- In this process excess ATP and NADPH are utilized thus prevents solarization.

**Measurement of photorespiration**

Air containing $CO_2$ is passed through an illuminated leaf placed in a glass chamber. The air then passes through an infra red gas analyzer which measures the amount of $CO_2$ in the air after its passage over an illuminated leaf. This is compared with control in which the air is passed through non-illuminated leaf. This will give light induced $CO_2$ evolution.

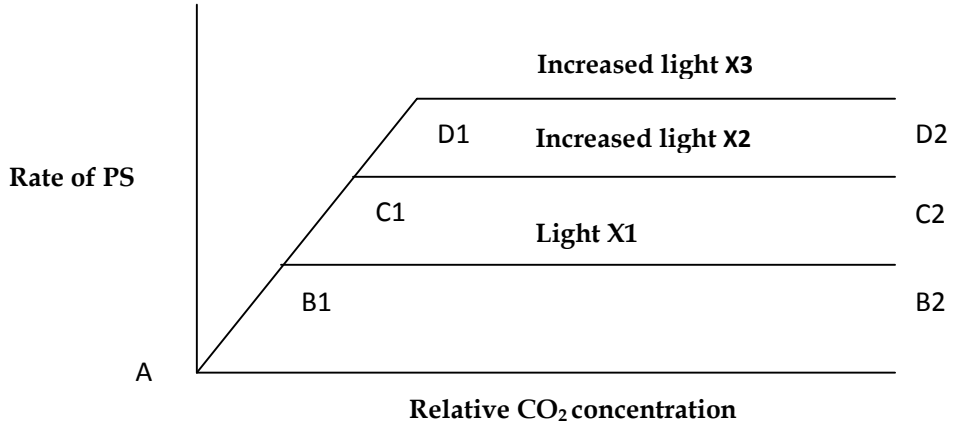
**Factors affecting the rate of photosynthesis** **Law of minimum:**

Leibig (1843) proposed the law of minimum. According to this when a physiological process is conditioned by several factors, the slowest factor controls the rate of process.

**Law of limiting factor:**

Blackman (1905) proposed the idea of law of limiting factor. According to this theory when a physiological process is going on as to its rapidity by a number of separate factors, the rate of process is limited by the pace of slowest factor. According to this theory when all the factors are available in abundance the least available factor controls the photosynthetic rate, but if the magnitude of this factor increases beyond the saturation point other factors become limiting. It means it is not fixed. Suppose one leaf is exposed to utilize a maximum of 5 mg of $CO_2$ / hr., but only 1 mg is available. $CO_2$ becomes the limiting factor. At this stage increase of $CO_2$ supply to 2 mg / hr. will increase the rate of photosynthesis. But when $CO_2$ is increased to 6 mg which is more than saturation point. At this stage the rate of photosynthesis can be increased by increasing the intensity of light. According to this graph the rate of photosynthesis increases from A to B1 when supply of $CO_2$ is increased to 5mg / hr. If $CO_2$ concentration is further increased the photosynthesis remains constant along B1- B2 because the light has become

limiting factor. At this stage if we increase the light intensity the photosynthesis rate increases from B1 to C1 then becomes constant along C1 - C2. If light intensity is further increased up to saturation point the rate of photosynthesis will increase from C1 to D1 and remains constant along D1-D2. Any further increase in light at this stage may not increase the rate of photosynthesis because some other factor would have become limiting.




**Law of relatively limiting factor**

Warburg, Boysen & Jensen (1918) stated that it is not necessary that the factor which is quantitatively smallest will act as limiting factor, but any factor whose quantity is less than the actual amount required will act as limiting factor. According to them in various chloroplasts where photosynthesis is going on limiting factors may not be the same.

**Factors external – Light, $CO_2$, temperature, water, $O_2$**

**Factors internal – Chlorophyll content, protoplasmic factors**

**Light – Intensity**

Rate of photosynthesis is maximum in diffused light. In bright light of noon photosynthesis declines. During day time rate of photosynthesis is ten times greater then respiration. At certain light intensity amount of $CO_2$ used in photosynthesis and amount of $CO_2$ liberated in respiration remains equal. This is called compensation point. In shade plants compensation point is retained for longer time. Light compensation point for heliophilic leaves is 100 -200 fc. Low light intensity causes closure of stomata which retards $CO_2$ entry in leaves thus reduces photosynthesis. Increase in intensity increases the photosynthetic rate but after certain limit it declines this is called saturation point. High light intensity retards photosynthesis due to

excessive transpiration and reducing water content of plant. It also causes photooxidation of chlorophyll molecule (Solarization) in present of $O_2$.

**Light quality**

White light between the range of 390 - 760 nm is most useful in photosynthesis. The maximum absorption by chlorophyll is done in the range of blue (440nm) and red (655nm). Photosynthetic bacteria can carry on photosynthesis even at 900 nm. Green light indicates least absorption.

**Duration of light**

When light intensity and other factors are favourable, the rate of photosynthesis is directly proportional to duration of light. Quantum yield is higher when plants are exposed to continuous day light of 10-12 hrs.

**$CO_2$**

The change in $CO_2$ concentration in atmosphere affects the photosynthesis (the concentration is .03 % of vol.). The sources of $CO_2$ are plant and animal respiration, bacteria in soil water and ocean, decay of organic substances and combustion of fuel. Godlewski (1873) found an increase in photosynthesis with increased $CO_2$ supply but up to a certain limit. The optimum level of $CO_2$ for maximum photosynthesis varies with plants. In *Triticum aestivum* it is 0.15 % and in water plant 1.1 %.

**Temperature**

Light phase of photosynthesis is unaffected by temperature but high temperature retards photosynthesis. The photosynthesis is possible even up to $60^0$C in exceptional case, but it increases up to $35^0$C. For every rise of $10^0$C temperature the rate of photosynthesis doubles (Q10-2). Extremes of temperatures cause injury or even permanent damage to plants. Cold temperature reduces photosynthesis by reducing enzymatic activities. The ice formation due to cold temperature changes the colloidal nature of cytoplasm. Ice formation also disturbs the permeability of membranes.

**Water**

Water scarcity causes dehydration, closure of stomata, inactivation of enzymes. Dehydration may cause irreparable damage to micro molecular structure of chloroplast membrane.

**$O_2$**

Accumulation of $O_2$ retards photosynthesis. Photosynthesis inhibition of $O_2$ is called Warburg effect. According to this both $CO_2$ assimilation and $O_2$ evolution are inhibited by atmospheric $O_2$. Gibbs demonstrated that high concentration of $O_2$ bring about competitive inhibition of Ribulose bi phosphate carboxylase. High $O_2$ concentration also inhibits photosynthesis by encouraging photorespiration.

**Internal Factors – Chlorophyll content**

Emerson (1929) found direct effect of chlorophyll content on photosynthesis in chlorella (fresh green water alga). Thomas (1955) found that photosynthetic rate is slow in young leaves and optimum in mature leaves. But leaves of old age show reduced rate of photosynthesis due to ageing effect during this time grana might be disintegrated.

**Protoplasmic factors**

The enzymes which take part in photosynthesis remain active only under hydrated conditions of cytoplasm. Therefore, hydration of cytoplasm is necessary.

**Nutrition**

Deficiency of mineral elements affects photosynthesis. Many elements like Fe and Mg influence the functioning of chlorophyll and cytochrome. Elements like Copper acts as co-factor of many prosthetic enzymes. Deficiency causes reduction in photosynthesis rate.

**Osmotic relations**

Osmotic relationship between different cells has indirect effect on photosynthesis as it plays a vital role in availability of water.

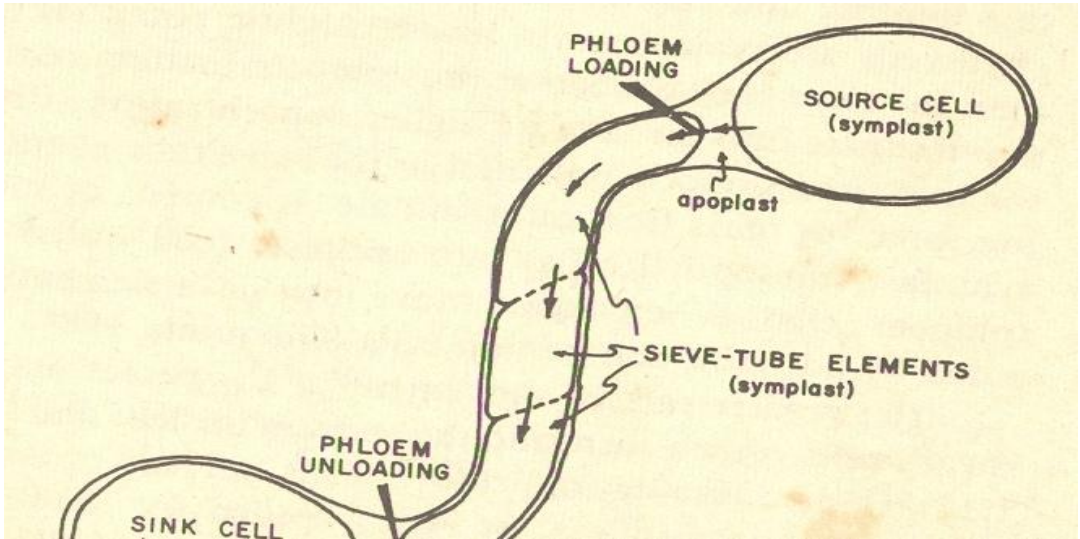
**Accumulation of end products**

Generally food manufactured during photosynthesis is translocated to other parts but if it remains in leaf it causes inhibition of photosynthesis. This is called end product inhibition.

**Phloem loading and unloading**

Phloem loading (transfer of photosynthates from the mesophyll cells of the leaf to the phloem sieve tube elements) and phloem unloading (transfer of photosynthates from phloem sieve tube elements to the cells of a sink) can be rate limiting and can affect translocation. During phloem loading the mesophyll cells are typically at a lower osmotic potential (higher water potential) than the sieve tube elements, thus the phloem loading requires an energy input to move sugars into an area of higher concentration. Phloem loading generates increased osmotic potential in the sieve tube elements, supplying the driving force for mass flow of assimilates. It consists of movement of sugars from simplast (mesophyll cells) into apoplast (cell walls) and then into symplast (phloem cells). When sugars move into sieve elements, the movement may be aided by adjacent companion cells (Giaquinta 1980). The greater rate of movement in $C_4$ species may be due to the vascular sheath cells which surround the veins and have chloroplasts. Under illumination, chloroplasts can help provide photosynthetic energy (ATP) needed for loading through sugar concentration gradient (Throughton and Currie 1977).

At the other end of the translocation process, phloem unloading can also limit the rate at which a sink receives assimilates. Some studies have shown that unloading is similar to loading in that the sugars move from the phloem symplast to the apoplast and then are transferred to the symplast of sink cells. However, there are indications that unloading may occur by a direct symplast transfer from phloem cells to sink cells (Mc Neil 1976). Current indications are that unloading occurs by different mechanisms in different tissues and may vary with the developmental status of the sink (Giaquinta 1980).



**Assimilate partitioning**

Partitioning of assimilates to the sink is generally higher when sinks are closest to the source. Upper leaves usually export to the shoot apex, lower leaves to the roots and middle leaves to both (Wardlaw 1968). For Example, the upper expending leaves of soybean will import more assimilates from the second leaf below them, which is on the other side of the stem (Thrower 1962). Cross linking of sieve tube elements occurs in most species, but some more efficient than others. Grasses have extensive cross linking at nodes, which essentially eliminates a preferred route by assimilate from any leaf to any particular sink (Gifford and Evans 1981). The photosynthetic source cell produces the sugars which can move symplastically to the sieve tubes. Phloem loading increases the sugar concentration of sieve tubes above that of the apoplast.

At the sink, carbohydrates are being absorbed and either actively partitioned into cell constituents (eg. starch) or changed to other carbohydrates that have little effect on hydrostatic pressure of the phloem. Phloem unloading lowers the concentrations of sugars in sieve tubes. The buildup of sugars at the source and the removal of sugars at the sink establish a hydrostatic pressure gradient which moves water and sugars from sources to sinks.

What are limitations of movement of assimilates from sources to sinks. According to mass flow hypothesis, anything increasing photosynthesis increases hydrostatic pressure and translocation rate . However, this is true only if sinks have the ability to utilize more assimilates. If they are unable to utilize the increased production there would be a steady build up of sugars in the system, causing a feed – back inhibition resulting in reduced photosynthesis (Mondal et al. 1978). Presumably photosynthetic rate would be reduced to the rate at which sinks could accept assimilate. For leaf photosynthesis to be at maximum potential rates, sinks must be able to utilize all the assimilates produced. Under these conditions partitioning would be controlled by sink strength, that is, sink availability and the rate at which available sinks can utilize assimilates (Gifford and Evans 1981).

Factors that control sink strength also control the partitioning in crop plants. The effect of hormones on enzymatic activity and the elasticity of sink cells can have a dramatic effect on partitioning. IAA, cytokinins, ethylene and gibberellic acid when applied to a cut stem surfaces cause assimilates to accumulate in the region of application (Gifford and Evans 1981). Hormonal influences on initiation, development and abortion of flowers and seeds have a significant effect

on source sink relationship in crops. Investigations revealed that hormones have indirect influence on translocation rates through affecting sink demand (Gifford and Evans 1981).

**Assimilate partitioning during vegetative growth**

Leaves and other green tissues are the original sources of assimilates. Some remains in the green tissues for cell maintenance and can be converted to starch or some other form of storage if translocation is slow. The rest is translocated to vegetative sinks which are composed for growth, maintenance and storage functions. During vegetative growth roots, stems and leaves are competitive sinks for assimilates. The proportions of assimilates partitioned to these three organs can influence plant growth and productivity. The investment of assimilates into greater leaf area development results in greater light interception. However, the leaves also require water and nutrients so investment in root growth is also necessary. Some crop plants such as most grasses have no stem during vegetative development and favour partitioning to leaves and roots.

Some meristems are in more favourable position to intercept assimilates. For example, the intercalary meristems of leaves are in a better position to intercept translocated assimilates than are the peripheral root and shoot meristems (Evans and Wardlaw 1976). Young developing leaves need imported assimilates to provide energy and carbon skeletons for growth and development until they produce enough assimilates to handle their own requirements. Thrower (1962), Webb and Gorham (1964) have shown that the leaves of soybean and squash are largely self sufficient when 50% of their final area is developed. After full expansion and under good environmental conditions for photosynthesis leaves may export 60 to 80% of their assimilates to other areas of the plant (Hofstra and Nelson 1969). As the leaf gets older and begins senescence it may fail to support its own energy requirements because of age or shading or both. Under these conditions the leaf does not export or import assimilates. Instead, cell maintenance requirements (respiration) are often greatly reduced which allows the leaf merely to survive. Before death many of the inorganic and organic compounds in the leaf are remobilized and translocated to other parts of the plant.

The early growth of branches and tillers requires importing assimilates from the main stem or other branches until they become autotrophic. In, oats this usually occurs between the two and four leaf stage (Labanauskas and Dungan 1956). Whether a branch or tiller becomes completely independent of the rest of the plant is variable among species. In timonthly, the tillers

behave as separate units once they become autotrophic (St. Pierre and Wright 1972). Little interaction between timonthly tillers occurs even under stress conditions, and roots are supplied only by the tillers to which they are attached. When under stress the autotrophic tillers of some species, such as rye grass (Marshall and Sagar 1968) and oats (Labanauskas and Dungan 1956), will again start transporting assimilates from the main culm. How partitioning of assimilates among tillers affects total yield is influenced by how much the additional leaf area of the tiller contributes to the total dry weight of the plant and how much the tillers contribute to harvestable yield, for example, tillers of maize do not usually produce grain.

**Assimilate partitioning during the reproductive phase**

Reproductive growth is often the primary part of the plant harvested for yield. Crops, whose flowers, fruits and seeds (and their products) are the economic yields, have been selected over time to partition large amounts of their total dry matter into reproductive parts. In such plants a large photosynthetic surface and supporting structures are required prior to fruiting. After flowering the reproductive sink becomes strong, which limits the assimilates partitioned for additional leaf, stem and root growth. In determinate species leaf and stem growth ceases at flowering, while indeterminate species have vegetative and reproductive growth occurring simultaneously. Thus intermediate species are variable in the relative strength of their vegetative and reproductive sinks. If there is much vegetative growth during vegetative development yield may be reduced. In determinate grain crops early growth is vegetative allowing the plant to intercept more light energy for photosynthesis as it increases in size and allowing for adequate water and nutrient absorption to support root growth. The number of leaves is established at the initiation of inflorescence and is affected by temperature and photoperiod. Shortly after seed initiation seeds become the dominant sink of annual plants. Therefore, during seed filling the major part of assimilates both current and stored is used for increasing seed yield.

**Assimilate partitioning during grain filling**

Photosynthates deposited in grain come from three major sources, current leaf photosynthesis, current photosynthesis from nonleaf parts and remobilization of assimilates deposited in other plant organs. How much each of these factors contributes to final grain yield is affected by species and environment. Work in wheat and barley has shown that the photosynthesis of flag leaf, stem and head which are the closest sources of the grain, is the primary contributor to the grain. Lower leaves supply the needs of lower stem and roots (Lupton

1966; Wardlaw 1968). The strength of the grain as a sink and the relative availability and strength of sources affect the assimilate partitioning. If the top layers are removed, the lower leaves will supply assimilates to the grain. If the lower leaves are removed, the flag leaf will transport assimilates to roots (Marshal and Wardlaw 1973).

It would be helpful to know just how much each source contributes to grain yield and variability involved. Porter et al. (1959) estimated that the contribution of preanthesis photosynthesis (remobilized assimilates) was 20%, current leaf and stem photosynthesis 45% and head photosynthesis 30%. Drought stress during grain filling reduces grain yield through reduced photosynthesis. Thus the sink demand for grain filling uses more remobilized stored assimilates which results in a much more proportional contribution by remobilization. Although remobilization is an important component of grain yield, photosynthesis during the grain filling period is normally the most important source of weight for grain yield. This is because most of the assimilates is used for that process. Since the heads of small grains are located at the top of the canopy in the best light conditions for photosynthesis and since the assimilates produced is next to the grain, head photosynthesis would be expected to contribute more to grain yield. Primitive wheat types have lower sink demands than modern wheat's. They rely primarily on head photosynthesis for grain yield, partitioning very little from leaves. Wheat developed with greater yield has an increased partitioning of photosynthates from upper leaves. Increasing photosynthesis of small grain heads could also increase yields, one way is to add awns (thin extensions of glume or lemma) which have been shown to double the photosynthetic rate of heads (Mc Donough and Gauch 1959). The primary effect of awns on yield components is to increase kernel weight (Suneson et al. 1948). Lupton (1966) showed that the amount of assimilates from glumes and flag leaves partitioned to the seeds increased as grain filling progressed and that glumes which are closest to the seeds , partitioned a larger percentage of assimilates to seeds than did flag leaves. Awns have shown no yield advantage in humid climates possibly because of increased susceptibility to disease or lodging (Mckenzie 1972). In maize in which the ear is located in the middle of the stem almost all the assimilates produced is from leaves or sheaths. During grain filling the upper leaves distribute about 85% of their assimilates to the ear. Remobilization of stalk reserves in modern maize is not much different from that of primitive maize (Valle 1981). Stalk reserves are positively correlated with stalk strength, lower

reserves mean weaker stems favouring stalk rot disease organisms. Modern maize cultivars have not been selected for strong remobilization so the plants maintain resistance to lodging.

**Remobilization**

Once produced assimilates are transported to many areas in the plant. It can be transformed into many compounds, some of them structural compounds such as cellulose, hemicellulose that provide for the physical structure of the plant and usually remain where they are synthesized. Plant cells do not have energy systems to degrade structural compounds, but many storage compounds that can be changed back into forms that can be translocated to other parts of the plant are also produced. The storage compounds are significant in maintaining growth and development constancy despite photosynthetic fluctuations and are mostly composed of carbohydrates but often include significant amounts of lipids and proteins. The movement of compounds from an area where they are once deposited to an area where they can be reutilized is referred to as remobilization. During certain phases of development more assimilates are being produced than is used in growth and development and this axis can be directed to storage compounds. At a later phase like fruiting when photosynthesis is not able to furnish the assimilates requirement of sinks , storage compounds can be remobilized and moved to active sites such as seed development .Remobilization occurs with both organic and inorganic compounds. During leaf senescence carbohydrates, nitrogenous compounds, phosphorus, sulphur and other mobile elements are remobilized and translocated to current plant sinks. During the heading and flowering stages of the plant the assimilates produced by the photosynthesis is more than is required by these processes. The extra assimilates are moved to the stem and stored primarily as starch. However, as the plant goes into grain fill starch is converted to sugars and translocated to the filling grain.

**Harvest index**

Two usual terms used to describe partitioning of dry matter by the plant are biological yield and economic yield. The term biological yield was proposed by Nichiporovich (1960) to represent total dry matter accumulation of a plant system. Economic yield and agricultural yield have been used to refer to the volume or weight of those plant organs that constitute the product of economic or agricultural value. The proportion of biological yield represented by economic

yield has been called harvest index, the coefficient of effectiveness or migration coefficient. All these terms characterize the movement of dry matter to the harvested part of the plant.

The harvest index can be represented as per specifications of Synder and Carlson (1984) as follows:

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Crop yield can be increased either by increasing the total dry matter production in the field or by increasing the proportion of economic yield or both. There is a potential for increasing yields by both methods. In oats (Tanaka and Fery 1976) a large genetic population showed variability in both biological yield and HI. Oat lines with high biological yield and a HI of 40 to 50% showed the highest grain yield. Crosbie and Mock (1981) showed that increase in both biological yield and HI were responsible for increased grain yield of three maize populations. In some grain crops the increase in seed yield has been due to increases in HI. In other words, the plants are not producing any more dry matter but are rather partitioning more of their dry matter into seed yield.

**Source and sink relationship**

The leaves formed in the initial stages also works as sink as they withdraw the nutrition from other parts of plants, but very soon they start producing the dry matter or photosynthates through photosynthesis and become source. Soon after the formation the young leaves begin to export carbohydrates through vascular tissues from other parts of plant into their veins. Simultaneously, they start producing the food material that is also accumulated in veins .As a result of this the osmotic pressure of cells of leaf veins becomes higher and water is withdrawn from the surrounding cells increasing the turgor pressure in cells of leaf veins. During this time the flow of assimilates from the leaves start and leaves become source instead of sink. Sieve elements of phloem mainly take part in transport of assimilates from source to the sink. The gradient of decreasing phloem turgor pressure from the source to the sink drives the flow. Example, in wheat cultivar Kalyan sona with the formation of grains at about 60 days after sowing the leaves start transporting the dry matter to the grains and grains act as sink. Initially the translocation starts at a slow speed but after some time the transportation rate becomes very high. In later growth phases rate of translocation becomes reduced. Dry matter translocation also depends upon the demand. More the dry matter lost through respiration by grains higher will be the translocation rate. The accumulation of dry matter into sink stops at physiological maturity

stage as the vascular connection to the seeds / grains from the source is broken by formation of abscission layer. The stage is marked by maximum seed dry weight. During grain filling stage the dry matter in leaves show a decline followed by a corresponding increase in dry matter of grains. It has been proved from the experiments that much part of it moves to the developing sink particularly from the upper leaves. In some crops or crop varieties other parts also act as sink as they also withdraw some part of dry matter from the leaves. Flag leaf in cereals provides more dry matter to the sink as compared to other parts. It has the advantage of being located near to the sink. It also receives more amount of solar radiation and subsequently produce more dry matter. In wheat the grain yield is determined by either supply of assimilates from source during grain filling or by the number and capacity of kernels to be filled by the source.